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Seasonal Mortality of Rainbow Trout (*Salmo gairdneri*) Planted in Small Eutrophic Lakes of Central Canada

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Ayles, G. B., J. G. I. Lark, J. Barica, and H. Kling. 1976. Seasonal mortality of rainbow trout (*Salmo gairdneri*) planted in small eutrophic lakes of central Canada. J. Fish. Res. Board Can. 33: 647–655.

The seasonal mortality pattern and the causes of the mortality of planted rainbow trout (*Salmo gairdneri*) were studied in eight small eutrophic lakes in central Canada used for extensive aquaculture. Two periods of mortality were revealed. The first occurred in all lakes during the first 60 days of residence in the lakes and resulted in the mortality of 60–90% of the numbers of fish stocked. The second period occurred in about 20% of the lakes and was due to low oxygen levels following the collapse of a bloom of *Aphanizomenon flos-aquae*. Rainbow trout are close to their physiological upper tolerance levels in these lakes. Possible environmental and biological causes of mortality during the first period are discussed.

Ayles, G. B., J. G. I. Lark, J. Barica, and H. Kling. 1976. Seasonal mortality of rainbow trout (*Salmo gairdneri*) planted in small eutrophic lakes of central Canada. J. Fish. Res. Board Can. 33: 647–655.

Nous avons étudié l'évolution des mortalités saisonnières et leurs causes chez des truites arc-en-ciel (*Salmo gairdneri*) introduites dans huit petits lacs eutrophes utilisés pour une aquiculture intensive dans le centre du Canada. Nous avons découvert deux périodes de mortalité. La première se produit dans tous les lacs durant les premiers 60 jours de résidence et résulte en une mortalité de 60 à 90% des poissons introduits. La seconde période se rencontre dans environ 20% des lacs et est causée par de faibles teneurs en oxygène résultant de l'affaissement d'une floraison d'*Aphanizomenon flos-aquae*. Les truites arc-en-ciel se trouvent près de leurs niveaux supérieure de tolérance physiologique dans ces lacs. Nous discutons des causes ambiantes et biologiques possibles des mortalités observées durant la première période.

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EXTENSIVE aquaculture of rainbow trout (*Salmo gairdneri*) is practiced in small eutrophic lakes on the Canadian prairies (Miller and Thomas 1956; Sunde et al. 1970). Trout are planted as fingerlings (3–4 g) in early May and grow, without supplementary feeding, to a marketable size (> 200 g) by the fall (Lawler et al. 1974). A small cottage type industry has developed (3700 farmers harvested an estimated one-half million fish in 1974) but has been impeded by variable survival of fish. Survival rates have ranged from 0 to 85% and average about 25–30%. Complete or partial summerkill, or the mortality of trout following the collapse of an algal bloom, has been named as the major factor affecting survival (Lawler et al. 1974). The purpose of this study was to determine the extent and cause of such fish kills and to identify other periods and causes of mortality.

The variability of survival from lake to lake and from year to year has precluded any determination of maximum or optimum long-term productivity of fish biomass or the determination of any relation between such production and any limnological parameters.

Experimental Methods

The geography of the study area (Sunde and Barica 1975) and general methods used in extensive pothole aquaculture (Lawler et al. 1974; Johnson et al. 1970) have been described previously. Based on past fish-stocking history and winter water chemistry analysis (Barica 1975a) four high-risk summerkill lakes and four low-risk control lakes were selected for water chemistry, phytoplankton, and fisheries studies in 1974 (Table 1).

Limnological conditions were monitored weekly from mid-May to mid-September and daily during a summerkill period. Samples were taken in a vertical profile at 1-m intervals at the center of the major basin of each lake. Dissolved oxygen was determined by modified Winkler method, total ammonia-nitrogen (i.e. the sum of $NH_4^+ + NH_3$ expressed as N) by phenol–hypochlorite method and chlorophyll *a* fluorometrically after extraction in 90% acetone (for details of analysis see Stainton et al. 1974). The ratio of un-ionized ammonia (NH_3) to total ammonia increases with increasing temperature and pH. The un-ionized free ammonia levels were calculated from total ammonia concentrations and ionization constants using Trussel's (1972) chart. Temperature was measured with a YSI Model 43-TR thermometer and pH with a Radiometer Model 4 pH meter. Continuous monitoring of surface and bottom water temperatures was carried out in L. 318 and L. 885 using Weksler recording thermometers.

Phytoplankton samples were taken at least monthly in all of the lakes and more frequently in lakes experiencing extensive algal blooms. The phytoplankton enumeration and sampling method (Kling and Holmgren 1972) and the phytoplankton successions in L. 885, L. 882, and L. 255, and species distributions in the study area in 1973 (Kling 1975) have been described previously.

Seasonal trout mortality was determined by observing the survival of trout in holding cages, survey gillnetting captures, and mark–recapture population estimates. The fish were raised at the Freshwater Institute's Rockwood Experimental Hatchery and transported the 240 km to the study area by truck. The maximum difference between tank and lake temperature was 2 C. Trout were stocked as 2.5-g fish between May 15 and 29, 1974, and harvested with gillnets in mid-October. The final harvest is a good measure of the total survival of fish (Ayles 1975). Because they were used for other studies as well, it was not possible to have exactly the same stocking rate in all lakes. However, stocking rate seems to have little effect on growth or survival within the ranges used in this experiment (Lawler et al. 1974). In the holding cage study, three covered 60 cm × 60 cm × 120 cm soft mesh cages, each containing 50 fingerlings, were suspended from the surface in widely separated areas of each lake at stocking. The number of fish in each cage was counted every 2 wk. The cage counts provided a continuous monitoring of the suitability of the lake waters for trout survival (caged fish grew to an average of 33 g by fall without feeding). A single survey gillnet of six mesh sizes was set in each lake every 2 wk to provide a qualitative measure of the number of fish in the lake. The size of the populations in high-risk lakes 885 and 587 and low-risk control lakes 721 and 318 was estimated monthly. The modified single census Petersen method was used for estimating population size and confidence limits of the estimate (Ricker 1958). One lake was done each week; fish were trapped for 2 days, marked with a hot wire brand and released, then retrapped for the next 2 days.

It has been known for some time that certain blue-green algae, including *Aphanizomenon* sp., liberate substances that can be toxic to fish (Baslow 1969). A simple static bioassay was performed to see whether an algal toxin could be implicated as the primary cause of the fish kills. At the time of an algal collapse eight containers of water, each holding five trout from a holding cage in another lake were set up, four containers from the summerkill lake and four from a control. Two of the containers from each lake were oxygenated and two not oxygenated. Mortality of trout in the oxygenated water from the summerkill lake would indicate that some factor other than low oxygen was a contributing factor to death of the fish.

Results

FISH MORTALITIES FOLLOWING AN ALGAL COLLAPSE

Two of the high-risk lakes, L. 885 and L. 587, had collapses of substantial blooms of blue-green algae and associated trout mortalities.

Midsummer algal blooms and collapses have been observed on L. 885 for the last 4 yr. In 1974 (Fig. 1) Chlorophyta and Diatomeae predominated in the spring, giving way to Cryptophyceae and then to *Aphanizomenon flos-aquae* in mid-June. At the time of the maximum algal biomass and maximum chlorophyll *a*, *Aphanizomenon* composed 99.4% of the algal population. The pattern of events immediately before, during, and after an *Aphanizomenon* collapse has been described in some detail (Barica 1975a). In 1974 the bloom collapsed in early August (Fig. 1), reaching a minimum of 3.8 μg/liter of chlorophyll *a* by August 10 as compared to the bloom peak of 106 μg/liter 11 days previously. The collapse was followed by a rapid drop in dissolved oxygen to 1.9 mg/liter on August 12 and 1.1 mg/liter on August 13 and a considerable release of ammonia to over 200 μg/liter of toxic un-ionized ammonia (24-h LC50; see Discussion). The mark–recapture population estimate (Fig. 1) was 750 fish on August 7 and counts of caged fish and test gillnetting indicated that there had been no substantial mortalities by August 9. By August 12 virtually all caged fish were dead, and dead fish were observed on the lake. Including the final harvest, only 46 fish were caught after August 12 so approximately 94% of the fish alive in the lake prior to the algal collapse were killed by the summerkill condition (Table 2).

Water samples were taken from L. 885 and L. 318 during the fish kill and static bioassays performed. The test temperatures ranged from 19.2 to 20.2 C. Oxygen levels in unoxygenated L. 318 water were 5.9 mg/liter and 5.8 mg/liter at the beginning of the experiment and 2.6 mg/liter and 2.2 mg/liter in unoxygenated L.885 water. The mortality of fish after 12 h was:

	Replica 1	Replica 2
oxygenated L. 318 water	0	0
unoxygenated L. 318 water	0	0
oxygenated L. 885 water	0	0
unoxygenated L. 885 water	60%	80%

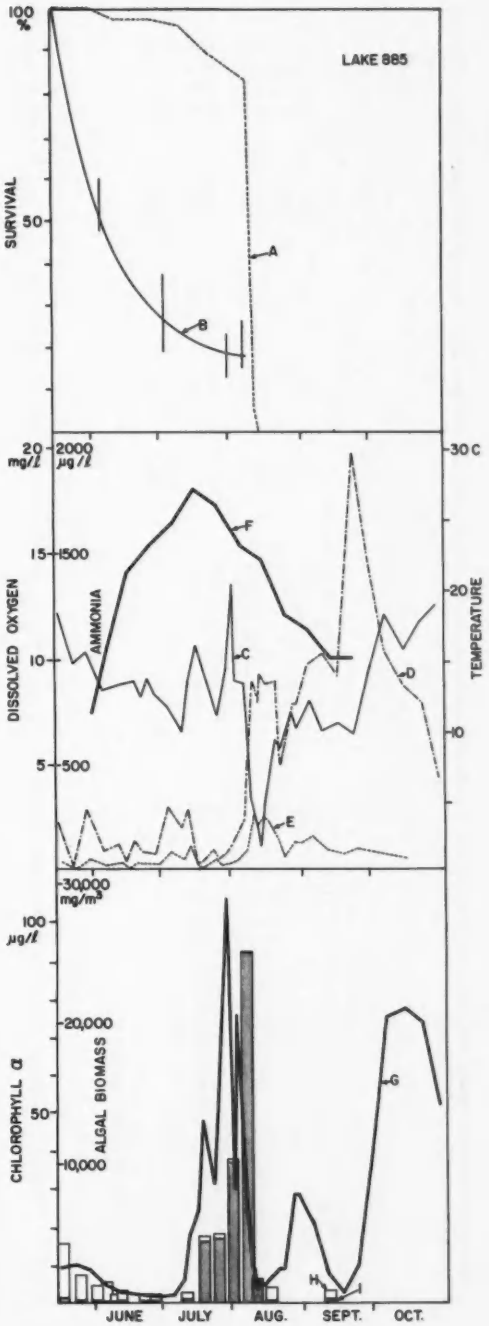

FIG. 1. L. 885. Results of fisheries, water chemistry, and phytoplankton studies in 1974, including A, percent survival of trout in holding cages; B, estimated percent survival of trout in the lake with 95% confidence limits; C, dissolved oxygen in milligrams per liter; D, total ammonia in micrograms per liter N; E, toxic un-ionized ammonia in micrograms per liter N; F, maximum weekly surface temperature in degrees C; G, chlorophyll *a* in micrograms per liter; H, total phytoplankton biomass in milligrams per m^3; and I, Cyanophyta biomass in milligrams per m^3.

TABLE 1. Morphological and chemical characteristics and stocking rates of four high-risk lakes and four low-risk control lakes in study area, 1974.

Summerkill risk	Lake	No. fish stocked	Area (*ha*)	Max. depth (*m*)	Mean depth (*m*)	Spec. cond. (*μmho/cm*)	Major ions[a] (*mg/liter*) Mg	Ca	SO_4	HCO_3	Secchi disc transp. (*m*)
High	400	9000	6.1	2.7	1.7	1835	176	75	834	530	0.3–2.6
	587	3400	6.9	4.0	2.5	2245	265	46	1117	570	0.5–3.6
	882	8400	5.7	3.4	1.5	2980	330	85	1522	620	0.3–2.2
	885	3600	2.4	3.0	1.9	1465	129	40	512	570	0.2–2.8
Low	675	14400	9.7	4.3	2.6	1940	208	83	948	580	0.5–2.5
	318	10800	21.9	2.4	1.4	795	63	56	259	385	0.7–2.2
	255	8000	3.2	2.7	1.7	815	78	39	269	335	0.6–2.7
	721	3200	6.5	3.0	1.6	3620	430	80	1926	697	0.8–2.8

[a]July–August 1974.

TABLE 2. Maximum values of certain environmental variables which approach the lethal levels for rainbow trout (*Salmo gairdneri*) in pothole lakes in 1974.

	Max. pH	Max. temp (*C*)	Max. total ammonia (*μg/liter N*)	Max. un-ionized ammonia (*μg/liter N*)	Min. D.O. (*mg/liter*)	Max. chlorophyll *a* (*μg/liter*)	Carbonate alkalinity (*mg/liter* $CaCO_3$)	Observed fish kills	Final survival
High-risk lakes									
400	9.0(224)[a]	23.2(203)	512(266)	35(266)	6.6(183)	134(135)	1043	No (60%)[c]	11.4%
587	9.2(219)	24.3(204)	571(209)	124(208)	.1(207)[b]	111(190)	1121	Yes (208)	7.9%
882	9.1(205)	23.6(198)	1058(247)	114(247)	3.6(247)	132(220)	1219	No (94%)[c]	24.5%
885	9.4(210)	24.8(194)	2187(267)	256(221)	1.1(225)[b]	106(210)	1121	Yes (224)	1.3%
Low-risk lakes									
255	9.5(218)	24.0(196)	831(271)	49(238)	6.9(224)	85(226)	1141	No	39.5%
318	9.2(136)	23.5(196)	326(203)	29(203)	3.7(203)	22(218)	757	No	10.7%
675	9.3(150)	24.3(198)	187(261)	23(226)	5.7(220)	32(268)	657	No	15.7%
721	9.1(162)	24.1(205)	936(267)	68(247)	3.6(227)	73(297)	1371	No	9.1%

[a]Numbers in parentheses indicate day of year that level was reached.
[b]Less than 100 min LT50 for trout acclimated to 100% air saturation (Merkens and Downing 1957).
[c]Indicates percent of fish alive prior to the algal collapse that were killed by the anoxic conditions following the collapse.

Lake 587 was classified as a high-risk lake (Barica 1975b) but in 2 previous yr of observation it had not summerkilled. In 1975 (Fig. 2) a mixed bloom of Diatomeae and Cryptophyceae in mid-May gave way to a mixed bloom of 99% *A. flos-aquae* and *Anabaena circinalis* by mid-July. The algal collapse in L. 587 was somewhat different from that of L. 885 in that the dead and dying algae settled out of the water column quickly and a layer of oxygenated water remained near the surface for 4 or 5 days. This difference may have been because L. 587 is considerably deeper than L. 885. A strong wind on the night of July 25 apparently stirred up the lower waters of the lake and on July 26 the dissolved oxygen levels had fallen to less than 0.1 mg/liter throughout the lake. Dead and dying fish were observed on the lake and the caged fish were all dead. When new cages of fish were put in, the fish died within an hour. Levels of toxic un-ionized ammonia did not exceed 200 μg/liter. Based on the final harvest and the mark–recapture population estimates it is estimated that 60% of the fish alive in the lake prior to the algal collapse were killed.

There were no periods of fish mortalities associated with the collapse of blue-green algal blooms in the low-risk lakes in which mark–recapture population estimates were made on a regular basis (Fig. 3 and 4). There were no significant algal blooms, and levels of dissolved oxygen did not fall below 3.5 mg/liter during the summer in either lake.

High-risk lakes L. 882 and L. 400 had significant blooms of blue-green algae but the blooms did not collapse precipitously. In all lakes, maximum pH, total and un-ionized ammonia, maximum temperature, minimum dissolved oxygen, and carbonate alkalinity approached levels which have been shown to be lethal to rainbow trout (Table 2) but were not associated with apparent fish mortalities (see Discussion).

Fish Mortalities not Associated with Collapses of Blue-Green Algae Blooms

The mortalities of caged fish (Fig. 5) in lakes

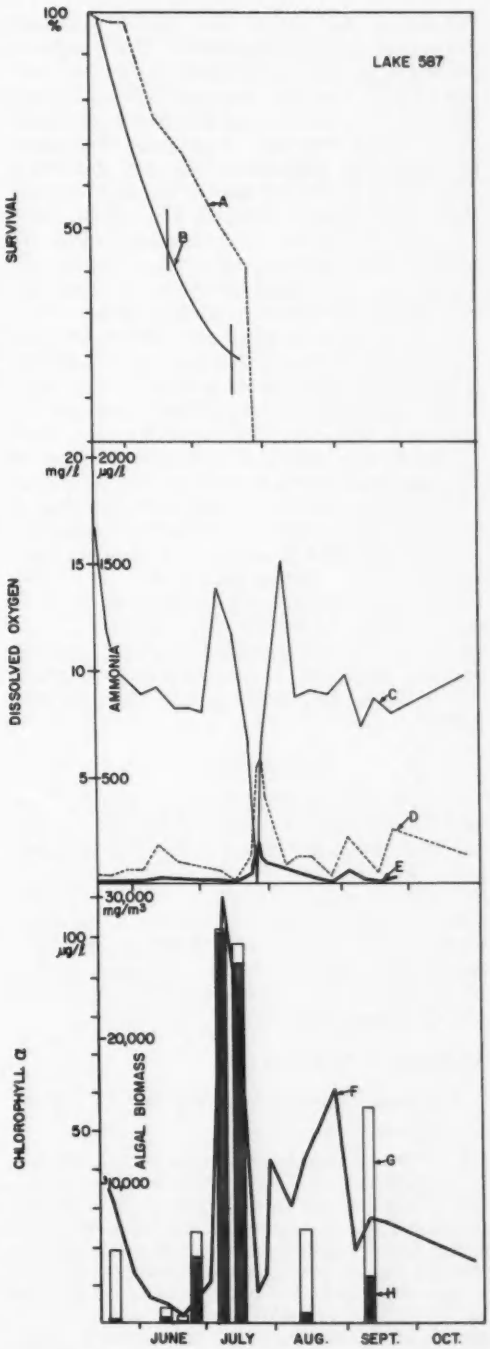

FIG. 2. L. 587. Results of fisheries, water chemistry, and phytoplankton studies in 1974, including A, percent survival of trout in holding cages; B, estimated percent survival of trout in the lake with 95% confidence limits; C, dissolved oxygen in milligrams per liter; D, total ammonia in micrograms per liter N; E, toxic un-ionized ammonia in micrograms per liter N; F, chlorophyll *a* in micrograms per liter; G, total phytoplankton biomass in milligrams per m^3; and H, Cyanophyta biomass in milligrams per m^3.

that did not summerkill were relatively constant throughout the growing season. There were no periods of significant mortalities except in L. 885 and L. 587. The final average survival of caged fish was 72%, and it is probable that at least some of the deaths were due to the stress of frequent handling. The gillnetting catch per unit effort (Fig. 5) (numbers of fish per 47 m of net per night) was highest during the first fishing period in mid-June. Catches in subsequent periods decreased until mid-July when they leveled off. There were no marked differences in catch per unit effort after mid-July. These qualitative measures of a rapid decrease and then leveling off of population size in all eight lakes were confirmed by the quantitative estimates in five of the lakes. The mark–recapture population estimates for lakes 587, 885, 318, and 721 (Fig. 1–4) show that there were significant mortalities of trout in the spring and very early summer. Approximately 85% of the total mortalities from planting to harvest in L.318 and L.721 occurred in the first 30 days and 96% occurred in the first 60 days. Mark–recapture studies in L. 255 after early August also demonstrated that little or no mortalities of fish occurred in late summer and fall. All of the environmental parameters measured reached maximum levels during midsummer (Table 2), a period when few mortalities occurred.

Discussion

Two periods of mortality of trout in these lakes have been revealed. Between 60 and 90% of the fish died during the first period which occurred in all lakes studied and lasted from stocking in mid-May till about mid-July. The second period occurred only in some of the lakes: it was associated with the collapse of a bloom of *Aphanizomenon flos-aquae*, and in the two lakes studied 94 and 60% of the fish present in the lake, prior to the collapse, were killed.

Summerkill Mortalities

The present results indicate that lethal dissolved

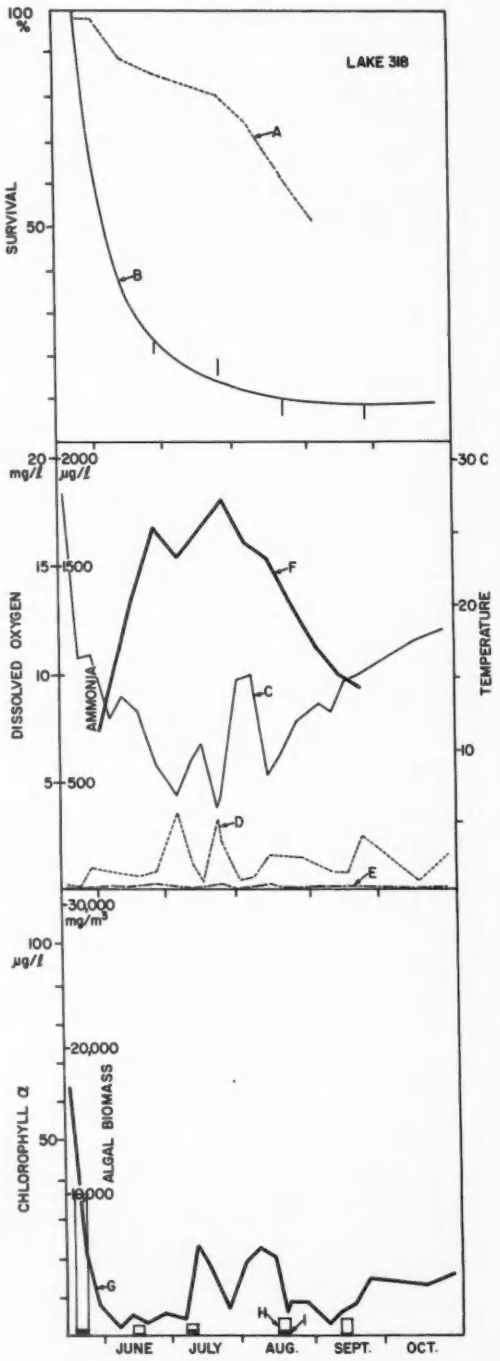

FIG. 3. L. 318. Results of fisheries, water chemistry, and phytoplankton studies in 1974, including A, percent survival of trout in holding cages; B, estimated percent survival of trout in the lake with 95% confidence limits; C, dissolved oxygen in milligrams per liter; D, total ammonia in micrograms per liter N; E, toxic un-ionized ammonia in micrograms per liter N; F, maximum weekly surface temperature in degrees C; G, chlorophyll *a* in micrograms per liter; H, total phytoplankton biomass in milligrams per m^3; and I, Cyanophyta biomass in milligrams per m^3.

oxygen levels were the primary cause of trout mortalities following an algal collapse. In L. 885 and L. 587 the oxygen levels were below the 100 min LC50 of 1.8 ppm reported by Merkens and Downing (1957) for rainbow trout acclimated to 15.2 C. Mackenthun et al. (1945) and Prescott (1948) found that toxic substances released by the decomposition of blue-green algae resulted in fish mortalities in well-oxygenated water. The survival of fish in oxygenated L. 885 water in the bioassay test indicates that if an algal toxin was present it had no noticeable effect during a 12-h period. The water temperatures and levels of toxic un-ionized ammonia were high but were less than lethal levels reported in the literature (Charlon et al. 1970; Ball 1967). The resistance of salmonids to low oxygen levels is reduced significantly by increased temperature (Gibson and Fry 1954) and by the presence of a small concentration of ammonia (Merkens and Downing 1957). It is possible that the levels of algal toxins, ammonia, or temperature while not lethal may have acted synergistically with the low oxygen levels to produce the fish mortalities. The partial summerkill in L. 587 indicates that there were refugia in the lake that remained suitable for trout survival.

Water chemistry studies (Barica 1975a) and trout stocking studies (L. A. Sunde and G. B. Ayles unpublished data) indicate that 30–40% of the lakes in the study area are potential summerkill lakes and approximately one half of them will have an algal collapse and associated fish kill in any one year. Based on a correlation between winter ammonia levels and maximum summer chlorophyll *a*, a method has been developed for predicting which lakes are likely to have only small algal blooms and are thus a low summerkill risk (Barica 1975b). The high-risk lakes can then be avoided for aquaculture purposes.

Nonsummerkill Mortalities

Lawler et al. (1974) held that most of the mortalities of trout planted in these lakes could be related to complete or partial summerkill. The present results show that the majority of mortali-

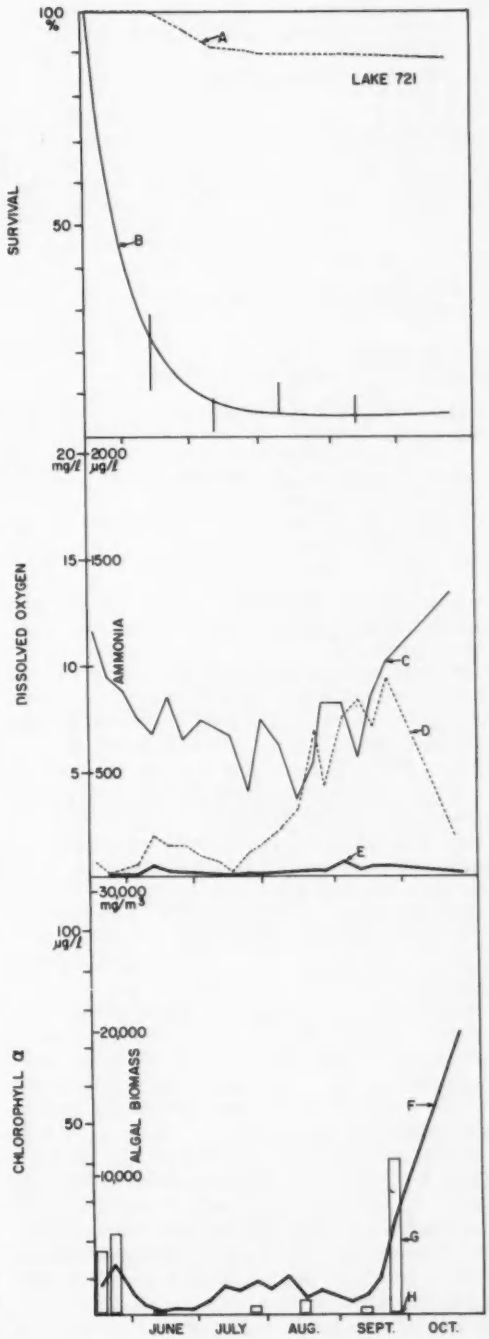

FIG. 4. L. 721. Results of fisheries, water chemistry, and phytoplankton studies in 1974, including A, percent survival of trout in holding cages; B, estimated percent survival of trout in the lake with 95% confidence limits; C, dissolved oxygen in milligrams per liter; D, total ammonia in micrograms per liter N; E, toxic un-ionized ammonia in micrograms per liter N; F. chlorophyll *a* in micrograms per liter; G, total phytoplankton biomass in milligrams per m^3; and H, Cyanophyta biomass in milligrams per m^3.

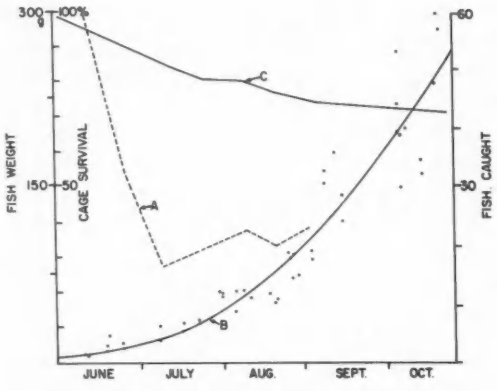

FIG. 5. Summary of fisheries studies from all eight experimental lakes (L. 885 and L. 587 deleted after summerkill), including A, average one-night catch in the survey gillnet; B, the average growth rate based on a second order polynomial fit of all fish caught during the summer; and C, the average percent survival in the holding cages.

ties in all lakes occurring shortly after planting are the result of unknown causes rather than algal summerkills.

Several environmental variables reached levels that are known to be stressful to trout, and conditions in the lakes are near the limits of the habitat requirements of rainbow trout. McCarraher (1971) reported that few fish species survived in alkaline eutrophic lakes of Nebraska when the carbonate alkalinity exceeded 300 mg/liter. The levels in these lakes were considerably higher, ranging from about 660 to 1400 mg/liter; however, the carbonate alkalinity in the Nebraska study was mostly as compounds of Na_2CO_3 and K_2CO_3 whereas Mg and Ca salts (as SO_4^{--} and HCO_3^-) predominate in the Erickson–Elphinstone area. The pH levels averaged less than 9.0 which is the level Jordan and Lloyd (1964) felt would be harmful to rainbow trout after exposure for several months but maximum pH levels generally lasting only 1 or 2 days did approach the 15-day LC50 of 9.5 (Jordan and Lloyd 1964). The maximum temperatures in the lakes, which occurred in mid-July, approached the 24-hour LT50 (25.5–26.0 C) (Charlon et al. 1970) but maximum temperatures lasted for only a short time and mean daily temperatures were usually 1–2 C lower. Levels of toxic un-ionized ammonia were in excess of the lowest 24-h LC50 for 200 μg/liter reported in the literature (EIFAC 1970). Ball (1967) found the asymptotic LC50 value to be 410 μg/liter and that the toxicity of un-ionized ammonia to trout decreased as the temperature rose and following acclimation. The above discussion of the possible critical environmental factors has not been exhaustive and there are certainly interactions between the various factors and their effect on the fish that have not been considered. However, in this study the lack of mortalities of caged fish, except during summerkill, clearly means that the physical environment of the lakes was suitable for trout survival throughout the season and other factors were causing the trout mortality.

We suggest that bird predation is a major cause of the early season mortality. Circumstantial evidence has suggested that predation by birds can drastically reduce the survival of planted salmonids in some lakes and streams (Johnson and Hasler 1954; Fraser 1972; Elson 1962). Common terns (*Sterna hirundo*) and Forster's terns (*S. forsteri*) migrate through Manitoba in early spring. Flocks of terns, including one of about 40 terns for 2 wk on L. 318, were seen on some of the lakes in May and early June in 1974, and three terns shot on L. 318 and two from another lake had been feeding on trout. The trout are particularly susceptible to predation shortly after stocking, and in several instances fingerlings stocked in the pothole lakes have shown signs of severe stress, spinning and jumping erratically on the surface, which would attract predators to the lake. Pearson (1968) found that the mean weight of prey of the closely related Arctic tern (*S. paradisea*) fishing off the Northumberland coast was 2.2 g, the average size of the trout at stocking, and the maximum size about 12–16 g which was approximately the mean size of the trout after 30 days and the minimum size after 60 days when mortalities had virtually ceased (Fig. 5). Additional studies will be necessary to quantify the importance of predation and to identify other biological or physical factors operating in the lake.

Although these studies were directed specifically toward an extensive aquaculture operation we suggest that certain aspects, in particular the rapid mortality of fish released into an apparently hospitable environment, may have relevance to other programs in which hatchery fish are released to the wild and returns are unexplainably low.

Acknowledgments

We gratefully acknowledge the assistance of the personnel of the Aquaculture project of the Freshwater Institute who participated in this project and those who thoughtfully reviewed the manuscript.

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Aspects of the Biology and Morphology of the Snake Eel, *Pisodonophis cruentifer* (Pisces, Ophichthidae)[1,2]

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WENNER, C. A. 1976. Aspects of the biology and morphology of the snake eel, *Pisodonophis cruentifer* (Pisces, Ophichthidae). J. Fish. Res. Board Can. 33: 656–665.

Variability in meristic and morphometric characters and dentition of the snake eel, *Pisodonophis cruentifer*, is presented. *Pisodonophis cruentifer* is distributed from the Laurentian Channel in the north to just north of Cape Hatteras in the south and is most abundant between 150 and 400 m in the Norfolk Canyon area. Males collected in June 1973 possessed mature spermatozoa in testicular tissue while females had yolk-laden eggs 0.6–1.0 mm in diameter. The total length–fecundity relation is $\log_{10} y = 4.28696 (\log_{10} x) - 7.33402$, where y = total fecundity and x = total length in millimeters. Gross and microscopic morphology of the gonads is presented. Morphological adaptations of *P. cruentifer* for burrowing into the substrate are contrasted with the benthopelagic eel, *Synaphobranchus affinis*, and actual burrowing into the substrate is documented.

WENNER, C. A. 1976. Aspects of the biology and morphology of the snake eel, *Pisodonophis cruentifer* (Pisces, Ophichthidae). J. Fish. Res. Board Can. 33: 656–665.

Nous décrivons la variabilité des caractères numériques et morphologiques, et de la dentition du serpent de mer, *Pisodonophis cruentifer*. L'espèce est répartie depuis le chenal Laurentien au nord vers le sud jusqu'à juste au nord du cap Hatteras. Elle atteint son maximum d'abondance entre 150 et 400 m dans la région du canyon de Norfolk. Les mâles capturés en juin 1973 possèdent des spermatozoïdes mûrs dans leur tissu testiculaire, alors que les femelles ont des oeufs chargés de vitellus d'un diamètre de 0.6–1.0 mm. La relation longueur total–fécondité est la suivante: $\log_{10} y = 4.28696 (\log_{10} x) - 7.33402$, dans laquelle y = fécondité totale et x = longueur totale en millimètres. Nous décrivons la morphologie macro- et microscopique des gonades. Nous comparons avec l'anguille bathypélagique, *Synaphobranchus affinis*, les adaptations morphologiques de *P. cruentifer* qui lui permettent de s'enfouir dans le substrat, et documentons le fouissement lui-même dans le substrat.

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Pisodonophis cruentifer is an ophichthid eel described by Goode and Bean (1896) from specimens trawled from 120 to 245 fath (220–448 m) in New England waters. Fowler (1918) placed *P. cruentifer* in his newly erected genus *Omochelys*, described for a species from the Phillippine Islands. *Omochelys marginatus* (Fowler 1948), an ophichthid trawled off the New Jersey coast, was later placed in the synonomy of *O. cruentifer* (Fowler 1952). Rosenblatt and McCosker (1970) referred *Pisodonophis* (with *Omochelys* as a synonym) to the subfamily Ophichthinae which are ophichthids that lack confluent dorsal, caudal, and anal fins. Kaup (1856a) used *Pisodonophis* in his original description of the genus while *Pisoodonophis* was used at a later date (1856b). This led to the use of the second spelling by Goode and Bean (1896) in their original description. Therefore, the presently accepted name for the snake eel is *Pisodonophis cruentifer* (Goode and Bean 1896).

Bigelow and Schroeder (1953) reported the species range from the type locales to waters off Cape Henry, Virginia, in depths from 24 to 245 fath (44–448 m). A northern extension to the Laurentian Channel was given by Scott and Crossman (1959). The eggs and larvae and their distribution in the Chesapeake Bight were described by Richardson (1974). Goode and Bean (1896) reported *P. cruentifer* as a parasitic boring form, a habit reiterated by Bigelow and Schroeder (1953) and Leim and Scott (1966) but dispelled by Walters (1955). Little else is known of the life history and ecology of this species. The purpose of this report is to document

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TABLE 1. Morphometric summary of *Pisodonophis cruentifer*. All values expressed as precents. CI = 95% confidence interval, A and B = the paratypes of Goode and Bean (1896), C = Fowler's (1948) type of *Omochelys marginatus*, TL = total length, PDL = predorsal length, PAL = preanal length, HL = head length, GL = gape length, HDE = horizontal diameter of the eye, LPFL = left pectoral fin length, TV = total vertebrae.

Character	Mean	*N*	±CI	Extremes	A	B	C
PDL/TL	12.1	116	0.2	9.7–14.9	13.6	14.4	12.8
PAL/TL	39.2	116	0.3	35.6–43.8	40.4	41.1	38.7
HL/TL	7.3	116	0.1	5.6–9.3	8.0	8.6	7.1
GL/HL	32.8	115	0.6	23.2–41.1	33.5	35.1	37.8
HDE/GL	28.6	116	0.8	18.6–44.0	29.8	27.5	27.1
LPFL/HL	28.1	68	1.0	22.1–40.2	27.7	25.5	32.9
TL					413	360	261
TV	148.4	53	0.7	138–154	147	148	150

meristic and morphometric variability in *P. cruentifer* and provide information on its life history and ecology.

Materials and Methods

Collections from a June 1973 cruise aboard the R.V. *Columbus Iselin* to the Norfolk Canyon area and the "open slope" area to the south of the canyon were used for life history, meristic, and morphometric studies. Thirty-minute tows with a 45-ft (13.7 m) shrimp trawl with a 0.5-inch (12.7 mm) stretched mesh codend liner were made in depth intervals of 75–150 m, 150–400 m, 400–1000 m, and 1000–2000 m. Data for latitudinal distribution were obtained from the R.V. *Albatross IV* groundfish survey which used a no. 36 Yankee trawl.

Morphometrics were taken according to Hubbs and Lagler (1958) with the exception of gape length, which is here defined as the distance from the tip of the snout to the angle of the jaws. The following counts were made on 47 specimens cleared and stained by the method of Taylor (1967): vertebrae (total, to pectoral insertion, to dorsal insertion, and to anal insertion), fin rays (left and right pectoral, dorsal, anal, and caudal), and left and right branchiostegals. The reduced first vertebra caudad of the skull was included in the counts, while all elements beyond the last hourglass-shaped centrum were counted as one vertebra. Tooth distribution patterns were drawn from 10 cleared and stained specimens with a camera lucida while those of the type specimens were done using a binocular microscope.

Fish utilized in reproductive analysis were measured to the nearest millimeter total length (TL). These were classed as immature when either the gonads were undifferentiated or, if differentiated, lacked gametogenic activity. Fish were gravid when eggs were large and easily separated from ovarian connective tissue or testicular lobes were swollen and milky white and milt was discharged with light abdominal pressure.

Ovarian tissue from 21 gravid females was removed and digested in Gilson's solution (Bagenal 1967). The freed eggs were strained through 202-μ plankton netting, washed in tap water, and blotted dry. Entire digested ovaries were weighed and either the total sample was counted or three replicate subsamples were taken, suspended in glycerin, and counted. Only the large eggs were included in the fecundity estimates. The longest horizontal diameter of 50 large and small eggs from each fish was measured with a calibrated ocular micrometer.

Gonadal tissue samples from 20 females and 10 males in several states of maturity were placed in Bouin's fixative at sea. Histological sections were prepared by standard techniques with Harris hematoxylin–eosin stains.

Results and Discussion

MORPHOMETRICS AND MERISTICS

Pisodonophis cruentifer is an elongate ophichthid with a nearly cylindrical body, ending in a hard pointed tail. The dorsal and anal fins are of low profile and originate slightly behind the pectorals and the vent, respectively. In fresh specimens they appear not to be confluent about the caudal. The head is acute with the upper jaw projecting beyond the lower jaw. The spatulate pectoral is of moderate size and inserts directly behind the rather small gill opening (see Goode and Bean 1896, figure 166). A summary of the morphometric characters is found in Table 1, and regression relations are in Fig. 1. Goode and Bean's type specimens (U.S. National Museum no. 28938) and Fowler's type of *Omochelys marginatus* (Academy of Natural Sciences of Philadelphia no. 72103) are included in the table for comparative purposes.

The meristic characters of *P. cruentifer* are variable (Fig. 2) as are those of other apodal fishes (Synaphobranchidae, Robins 1971; Anguillidae, Ege 1939). The most variable characters are dorsal and anal fin rays, which are related by the equation, dorsal fin rays = 1.05 anal fin rays + 80.4 ($r = 0.67$, $n = 35$). The positive

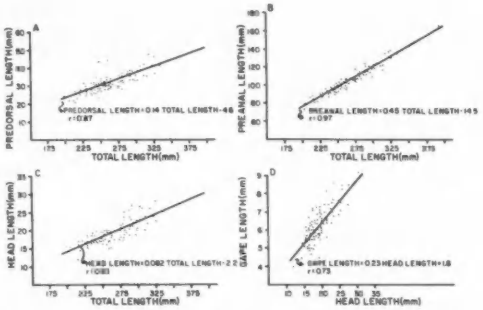


FIG. 1. Regression relations of various morphometric characters of *Pisodonophis cruentifer*.

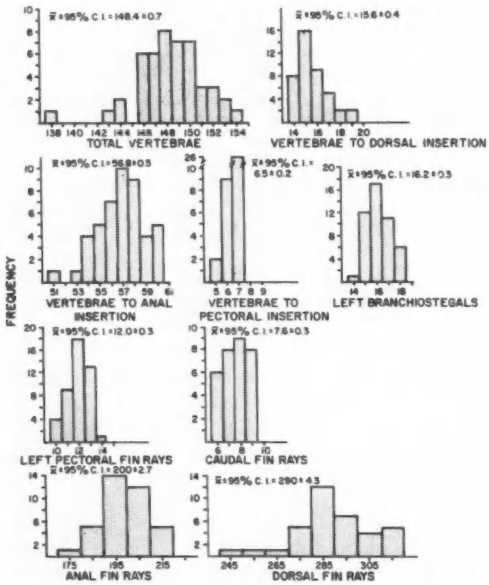


FIG. 2. Frequency distributions of meristic characters of *P. cruentifer*. C.I. refers to the 95% confidence interval.

correlation of number of D + A rays is also found in the American eel, *Anguilla rostrata* (Wenner 1972), and the synaphobranchid eel, *Synaphobranchus affinis* (Wenner 1975).

The dental formula of *P. cruentifer* is variable (Table 2) except for the ethmoid–premaxillary complex which had only five or six recurved teeth. Loss of teeth in some specimens (shown by empty sockets in certain cleared and stained specimens) accounts for some of the variability. An average dental formula (Fig. 3) based on 10 cleared and stained specimens plus the types of

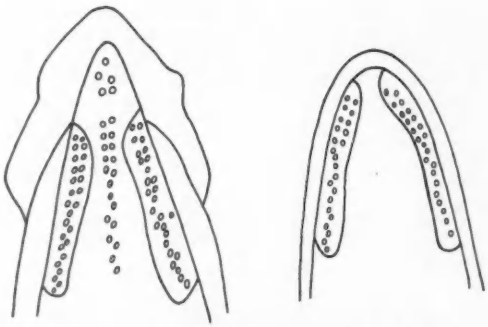


FIG. 3. Diagram of dentary, maxillary, ethmoid–premaxillary complex, and vomerine dentition in *P. cruentifer*. *Left*, upper jaw; *right*, lower jaw.

Pisodonophis cruentifer and *Omochelys marginatus* is: left and right dentary, 6 pairs of teeth followed by 13 in a row; left and right maxillary, 7 pairs of teeth followed by 12 in a row; ethmoid–premaxillary complex, 1 tooth followed by 2 pairs of teeth; vomer 5 pairs of teeth followed by 9 in a row. The ethmoid–premaxillary complex has the largest and the most recurved teeth and the uniserial teeth are not always aligned in a straight fashion on the bone.

DISTRIBUTION AND REPRODUCTION

Pisodonophis cruentifer is the most northerly occurring ophichthid eel on the Atlantic coast of North America (Fig. 4). Trawling operations in the Norfolk Canyon area revealed the following bathymetric distribution of the snake eel:

Depth interval (*m*)	No. caught	No. ½-h tows
75–150	32	12
150–400	87	14
400–1000	2	10
1000–2000	1	10

The shallowest capture of this species was at 36 m while the deepest was 1350 m, a bathymetric range of ca 1300 m. The possibility of the deep specimen being a washdown from a previous shallow water trawl is remote as the five tows preceding the capture were in depths greater than 1000 m.

Richardson (1974) described the eggs and larvae of *P. cruentifer* from plankton collections taken from July to September in waters overlying the Continental Shelf, 74–148 km from the Virginia coast. The eggs were greater than 2 mm in diameter and they were taken almost entirely in surface tows.

The R.V. *Columbus Iselin* cruise yielded 35

TABLE 2. Dental variation in the ophichthid eel, *P. cruentifer*, in nine specimens from Norfolk Canyon, plus Goode and Bean's types (1896) and Fowler's type of *Omochelys marginatus* (1948). CI = 95% confidence interval.

Character	Mean	±CI	Extremes
total length (mm)	277	30.8	245–413
left dentary teeth	24.8	1.8	21–29
right dentary teeth	24.7	1.6	20–27
ethmoid–premaxillary teeth	5.2	0.4	5–6
vomerine teeth	18.6	2.3	14–25
left maxillary teeth	26.5	3.3	17–37
right maxillary teeth	26.3	2.6	18–34

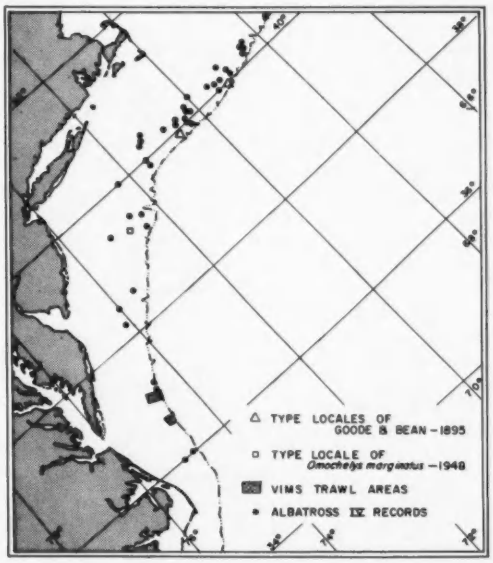


FIG. 4. Distributional map of *P. cruentifer*. Shaded areas are the Norfolk Canyon and "Open Slope" trawl survey areas.

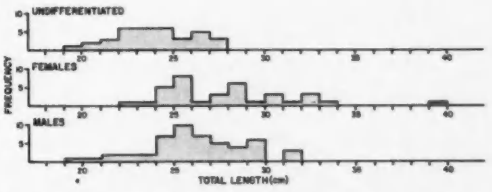


FIG. 5. Length frequency distribution of *Pisodonophis cruentifer* from June 1973, R.V. *Columbus Iselin* cruise.

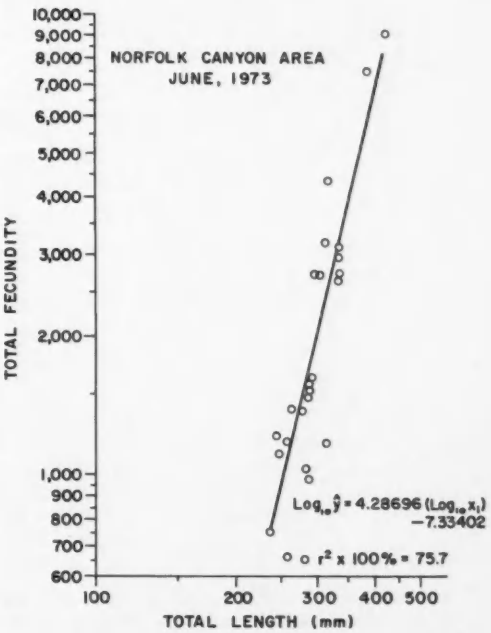


FIG. 6. Total length fecundity relation of *P. cruentifer* from the Norfolk Canyon and "Open Slope" areas, June 1973. y = total fecundity, x = total length.

gravid females ranging from 227 to 423 mm TL and 49 ripe males from 197 to 317 mm in the Norfolk Canyon area. The sex ratio of mature individuals did not deviate significantly from equality ($\chi^2 = 2.34$). Mature females were significantly larger ($t = 3.2$, df = 82), mean TL 278 mm, than mature males, mean TL 263 mm (Fig. 5).

As the American eel, *Anguilla rostrata*, approaches sexual maturity, the relative size of the eye is significantly larger when compared to immature forms (Wenner 1973; Wenner and Musick 1974). The same phenomenon occurs in the worm eel, *Ahlia egmontis* (Cohen and Dean 1970). Analysis of variance for arc-sine trans-

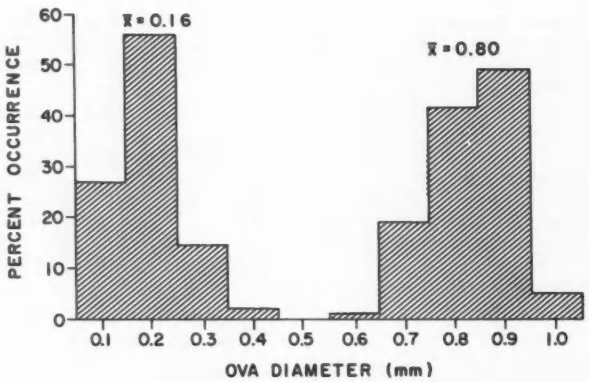


FIG. 7. Frequency distribution of egg diameters from *P. cruentifer* used in fecundity relation.

formed values (Sokal and Rohlf 1969) of the horizontal eye diameter expressed as percent of the gape showed no significant differences between mature males, mature females, and immature *P. cruentifer* ($F = 0.76$, df = 2,113). The increase in eye size of *A. rostrata* along with the shift to rhodopsin, a visual pigment associated with deep-sea fish (Wald 1958), and the increase in eye size of *A. egmontis* (the pigment has yet to be investigated) may reflect the movement of these two species into a different habitat, that of bathypelagic fish, with the onset of sexual maturity. The lack of changes in the eye of *P. cruentifer* may indicate that this species does not have a habitat change associated with reproduction.

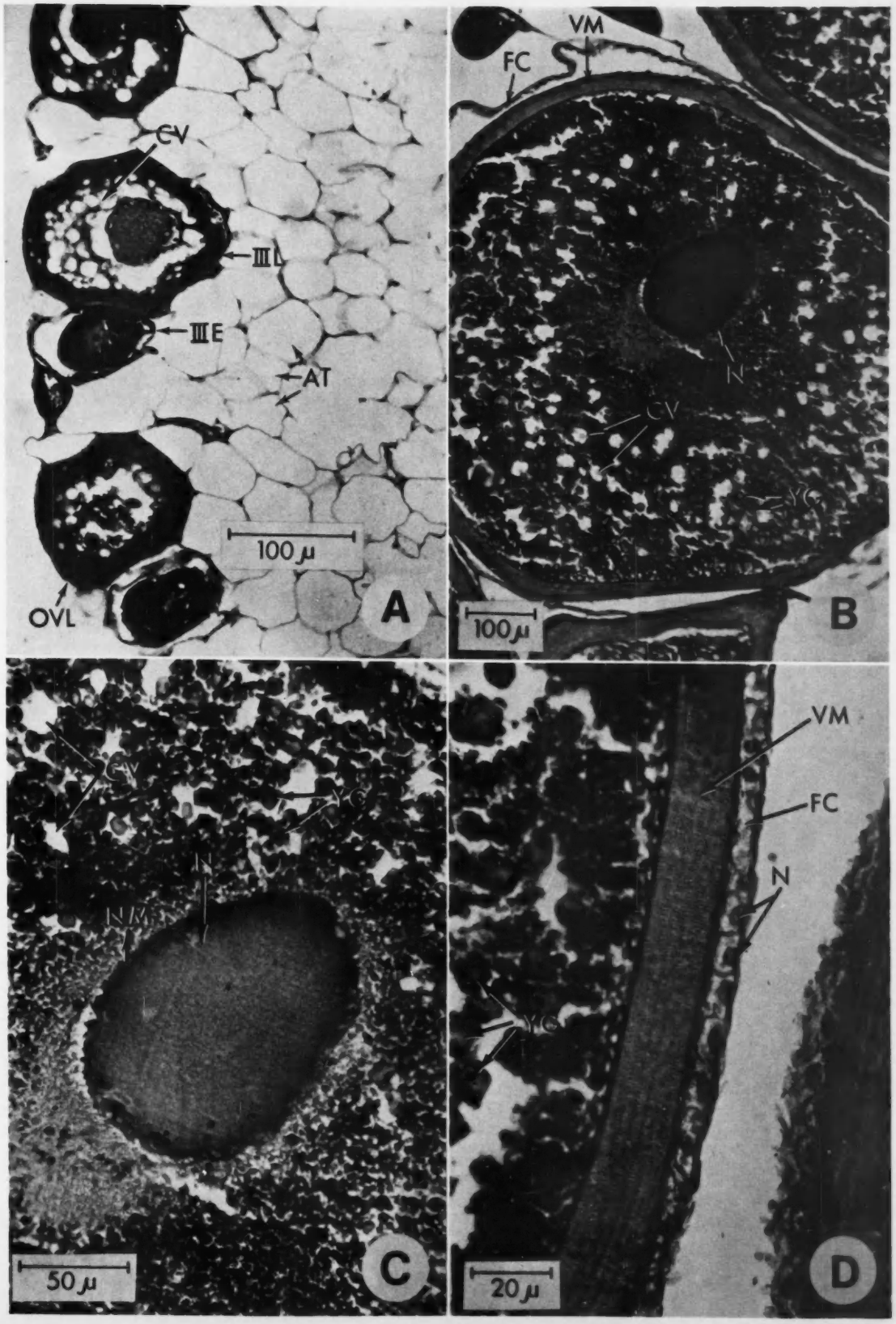
The relation between total length and fecundity for 21 specimens from 237 to 423 mm TL is $\log_{10}y = 4.28696\ (\log_{10}x) - 7.33402$, where y is the total fecundity and x is TL in millimeters (Fig. 6). The least number of eggs was 658 for a 283-mm fish and the greatest was 9013 for a 423-mm eel. The coefficient of determination ($r^2 \times 100$, Sokal and Rohlf 1969) showed that 75.7% of the variation in fecundity is attributable to changes in total length.

The longest horizontal diameters of the June oocytes fell into two nonoverlapping classes (Fig. 7). Full seasonal sampling must be conducted to determine whether two egg-size groups in ovarian tissue during June indicate multiple spawning.

The immature ovary is suspended from the dorsal peritoneum and has the appearance of a convoluted ribbon. Histological sections show early and late stage III oocytes (Combs 1969) aligned along the edge of the ovigerous lamellae (Fig. 8A). Early stage III oocytes have an average horizontal diameter of 0.11 mm and are characterized by an ovoid shape and densely basophilic cytoplasm. The oval, centrally located nucleus is densely basophilic and 0.05 mm in diameter. Oocytes between early stage III and late stage III have a dense basophilic cytoplasm and a basophilic nucleus. Late stage III oocytes have a mean diameter of 0.19 mm and are characterized by an irregular or sometimes convoluted cell membrane and lightly basophilic nuclei and cytoplasm. Although late stage III oocytes may have cytoplasmic vesicles, no yolk granules are present and egg membranes have not as yet developed.

In maturing females (Fig. 9A), the ribbon shape of the immature ovary is lost and the entire gut cavity is filled with oocytes which are easily dislodged from ovarian connective tissue. The right ovary terminates slightly caudad of the anus while the left ovary ends at about the level of the anus. The ovary from a gravid female contained large stage V oocytes (Fig. 8B) which had large quantities of eosinophilic yolk granules, cytoplasmic vesicles, and a spherical to slightly

FIG. 8. Histological sections of gonodal tissue from *P. cruentifer* collected June 1973. Bouin's fixative, Harris hematoxylin–eosin stain. (CV = cytoplasmic vesicle, IIIE = early stage III oocyte, IIIL = late stage III oocyte, AT = adipose tissue, OVL = ovigirous lamellae, N = nucleus, FC = follicle cells, VM = vitelline membrane, YG = yolk granules, NM = nuclear membrane, PVN = protovitellonucleoli, VD = vas deferens, S = spermatozoa.) A, cross section of ovarian tissue from a 255-mm immature female; B, Cross section of a developing oocyte from a 253-mm gravid female; C, Nucleus of oocyte in 8B; D, vitelline membrane and follicle cells surrounding oocyte in Fig. 8B.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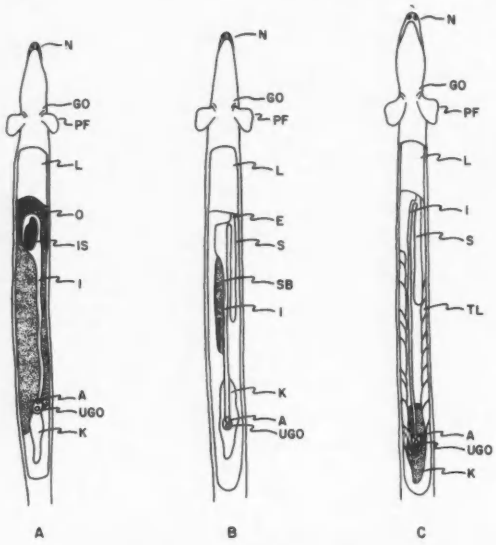


FIG. 9. Internal anatomy of *P. cruentifer*. A, Gravid female with eggs filling the peritoneal cavity (stipled area and circles represent the extent of the ovarian tissue within the cavity); B, Gravid female with the eggs removed to show the digestive tract and other structures; C, Mature male showing the extent of the testis. (*N* = anterior nostril, GO = gill opening, PF = pectoral fin, L = liver, IS = ingested sediment in stomach, I = intestine, A = anus, UGO = urogenital opening, K = kidney, E = esophagus, S = stomach, SB = swimbladder, TL = testicular lobes.)

elliptical nucleus near the center (Fig. 8C). The follicle cell layer (Fig. 8D) is made up of brick-shaped cells, 21 × 7 μ. The eosinophilic vitelline membrane underlying the follicle cells (Fig. 10A) is 20 μ wide. Basophilic stippling and the striated appearance of the vitelline membrane suggest a microtubular system for the transport of materials from the follicle cells into the developing oocyte. However, this can be borne out only by ultrastructural studies. The smaller oocytes (0.1–0.3 mm in diam) in the ovary of a maturing female are cuboidal (Fig. 10B) with basophilic inclusions around the inner periphery of the nucleus, the protovitello-nucleoli of Combs (1969).

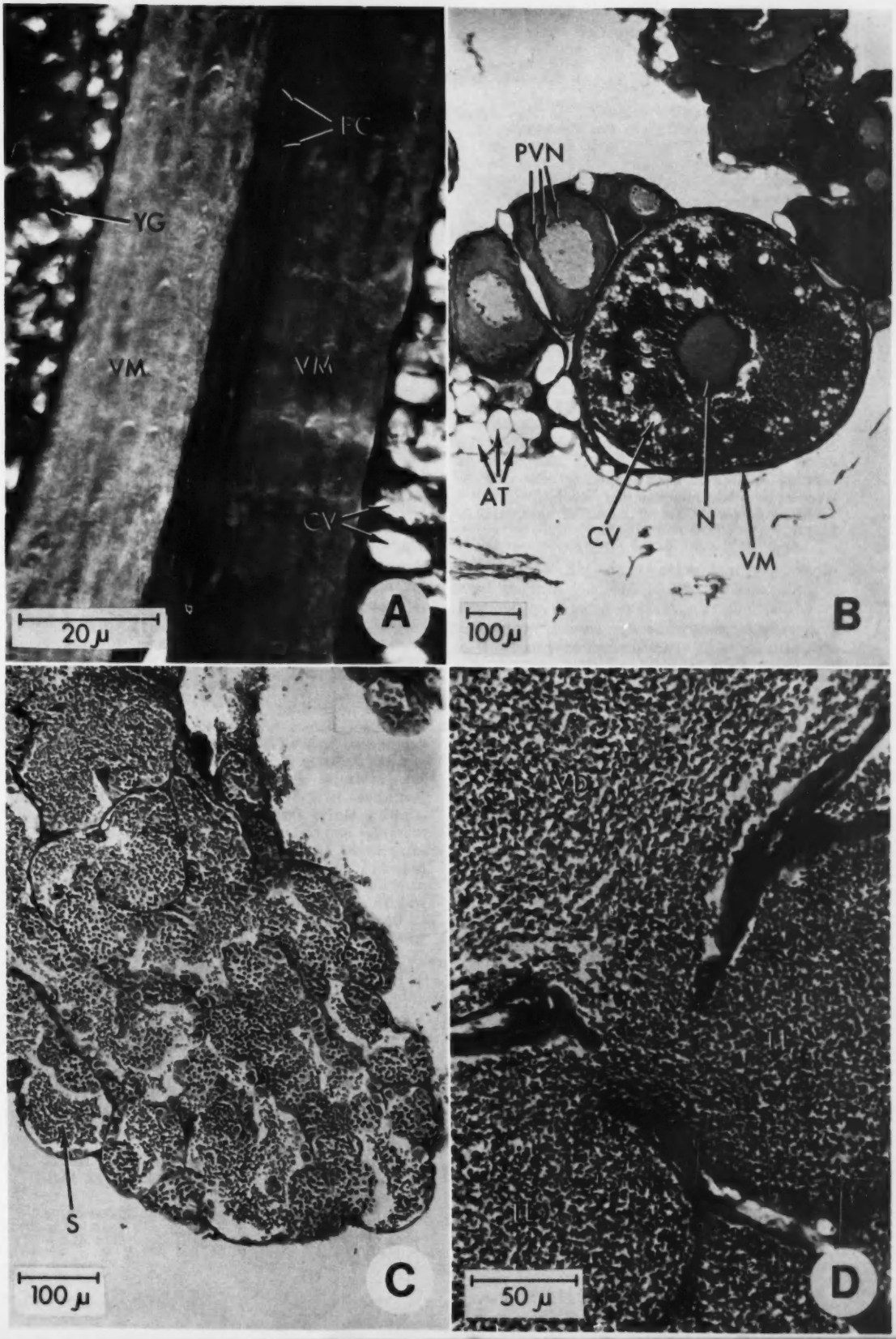
Immature males are rather difficult to sex due to the small indistinct nature of the testes at that stage. The paired testes are attached by connective tissue to the dorsal peritoneum. The left and right testes of mature males are unequal (Fig. 9C). The right testis is composed of about 10 unequally sized lobes extending from midpoint of the caecal stomach caudad to slightly behind the anus. The left testis originates near the end of the stomach and terminates near the vent and has about 8 lobes. A cross section of one of the testicular lobes is found in Fig. 10C. The vas deferens extends along the dorsal margin of the testis (Fig. 10D). Its exact relation with the urinary duct was not determined. The testis is composed of nests of gametogenic cells in a variety of stages ranging from primary spermatogonia to mature sperm.

HABITS AND FOSSORIAL ADAPTATION

Simenchelys parasiticus is a parasitic boring eel in the flesh of fishes and sometimes excavates large cavities (Goode and Bean 1896; Bigelow and Schroeder 1953). Goode and Bean (1896) stated "the peculiar and savage physiognomy of the fish (*P. cruentifer*) suggests at once the idea that it is a parasitic boring form." Walters (1955) pointed out that ophichthids, which were supposedly parasites, were found in the coelomic cavities of the "hosts" and convincingly argued that ingested ophichthids could rupture the alimentary canal with their pointed tail and enter the coelomic cavity where they become mummified. When the stomach contents of seven *S. parasiticus* and *P. cruentifer* were compared, I found white fibrous material which closely resembled fish muscle tissue in all *S. parasiticus* stomachs but none in *P. cruentifer*. The snake eel stomachs contained three small crabs (*Cancer* sp.), a sipunculid, and amphipod and polychaete remains. This provides some evidence that *P. cruentifer* feeds on benthic infauna and epifauna.

Ophichthid eels, particularly the better known tropical forms, are characterized by their ability to burrow tail-first into the sediment (Bohlke and Chaplin 1968). *Pisodonophis cruentifer* has morphological adaptations for a fossorial existence which include dorsal and anal fins of low profile,

FIG. 10. Histological sections of gonodal tissue from *P. cruentifer* collected June 1973. Bouin's fixative. Harris hematoxylin–eosin stain. Abbreviations same as Fig. 8. A, Cross section of two adjacent oocytes from a 288-mm gravid fish showing the vitelline membranes; B, cross section of gonadal tissue from a 243-mm female showing maturing and smaller immature oocytes. Note the rather irregular shape of the smaller oocytes and their lack of yolk granules; C, Cross section of a testicular lobe from a 240-mm ripe male showing the presence of spermatozoa in the seminiferous tubules; D, Cross section of gonadal tissue from a 243-mm ripe male showing the emptying of the ripe spermatozoa from the testicular lobes into the vas deferens.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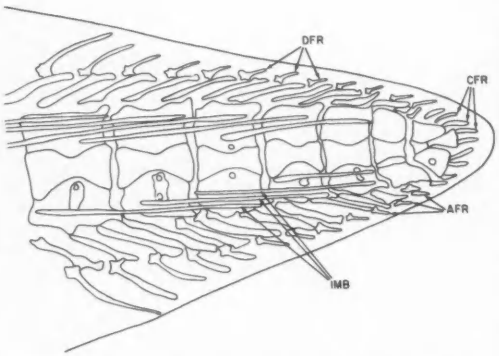

FIG. 11. Caudal osteology of a 243-mm cleared and stained *P. cruentifer* (VIMS collection no. 4331). Note reduction in size of the dorsal and anal fin rays as the tip of the tail is approached. The caudal fin rays are reduced to mere rudiments. Also note the presence of robust intermuscular bones (IMB). DFR = dorsal fin rays; CFR = caudal fin rays; AFR = anal fin rays.

moderately small pectorals and gill openings, and a stout, pointed tail. It does not alter its basic body shape even as sexual maturity is approached. Eels in general have a high fecundity (Wenner and Musick 1974) and in some species, such as *Synaphobranchus kaupi*, the abdomen becomes distended by the large number of eggs (100,000+ in a 600-mm TL fish) of over 1 mm diam (Wenner unpublished data). Relatively low fecundity and the related absence of abdominal distention in *P. cruentifer* insure the maintenance of a sleek outline for rapid penetration into the sediment. Consequently, reduced fecundity may be of selective value because of increased prespawning survival of females.

The adaptations of *P. cruentifer* for burrowing become even more apparent when the caudal osteology is compared to that of *Synaphobranchus affinis*. *Synaphobranchus affinis*, a benthopelagic eel (Robins 1971), has fairly large pectorals, high dorsal and anal fins which are confluent about the tail, and normal caudal fin rays (Robins 1971). The size of the dorsal and anal fin rays in *P. cruentifer* decreases to mere rudiments posteriorally and these, as well as the caudal fin rays, are detectable only in cleared and stained specimens (Fig. 11). The amount of bone in the tail when compared with the height of the tail is much greater in *P. cruentifer* than in *S. affinis*. These attributes and the presence of robust intermuscular bones decrease tail flexibility and provide an increased rigidity that enables the animal to rapidly penetrate the sediment.

The acual burrowing behavior of *P. cruentifer* was observed when a live specimen trawled from 280 m at 37°37′N, 72°01′W by the R.V. *Albatross IV* on March 14, 1974 was placed in an aquarium with several inches of sandy sediment. The animal was in excellent condition and repeated observations were made over a period of 4 days. The fish rested on the surface of the sediment with its body in an S-shaped curve. When burrowing, the fish backed into the substrate utilizing its tail to gain entrance. Rapid S-shaped flexures of its body followed until it was either partially or completely covered with sediment.

During dive number 574 of Deep Submersible Research Vehicle *Alvin* in Norfolk Canyon (submersible down: 37°03.3′N, 74°38.4′W; submersible up: 37°04.1′N, 74°38′W) from 1539 to 1941, *P. cruentifer* was observed in greatest numbers at depths from 250 to 350 m. The in situ burrowing behavior was not observed, but numerous individuals were seen with only their heads exposed from the sandy substrate or resting on the sediment with their bodies in S-shaped curves. The burrowing behavior and the slender body shape which could allow it to pass through coarse net meshes may explain the relative paucity of this species in trawl collections.

Acknowledgments

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Hepatic Biotransformation of Environmental Xenobiotics in Six Strains of Rainbow Trout (*Salmo gairdneri*)[1]

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PEDERSEN, M. G., W. K. HERSHBERGER, P. K. ZACHARIAH, AND M. R. JUCHAU. 1976. Hepatic biotransformation of environmental xenobiotics in six strains of rainbow trout (*Salmo gairdneri*). J. Fish. Res. Board Can. 33: 666–675.

Six strains of rainbow trout (*Salmo gairdneri*), each geographically and genetically distinct, were compared in relation to their capacity for hepatic hydroxylation of aniline and benzo[a]pyrene, and reduction of *p*-nitrobenzoic acid to *p*-aminobenzoic acid, in vitro. The effects of 3-methylcholanthrene on stimulation of nitro reductase and aryl hydrocarbon hydroxylase were examined. For the three systems studied, a significant pattern was observed with respect to each strain's capacity for metabolism. Induction experiments suggested that environment may influence gene expression. Enzyme kinetics and spectral properties were also examined, and mechanisms causing variability within and among strains were discussed.

PEDERSEN, M. G., W. K. HERSHBERGER, P. K. ZACHARIAH, AND M. R. JUCHAU. 1976. Hepatic biotransformation of environmental xenobiotics in six strains of rainbow trout (*Salmo gairdneri*). J. Fish. Res. Board Can. 33: 666–675.

Les auteurs comparent six lignées de truite arc-en-ciel (*Salmo gairdneri*), géographiquement et génétiquement distinctes, sous le rapport de leur capacité d'hydroxylation hépatique de l'aniline et du benzo[a]pyrène, et de réduction de l'acide *p*-nitrobenzoïque en acide *p*-aminobenzoïque, in vitro. Ils étudient les effets du 3-méthylcholantrène sur la stimulation de la nitro réductase et de l'aryl hydrocarbone hydroxylase. Dans les trois systèmes étudiés, la capacité de métabolisme de chacune des trois lignées suit un schéma significatif. Des expériences d'induction suggèrent que l'environnement peut affecter l'expression des gènes. La cynétique et les propriétés spectrales des enzymes ont également été examinées, et on discute des mécanismes responsables de la variabilité intra et interlignées.

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UNTIL 1962, reports indicated that fishes lacked the NADPH- and oxygen-requiring microsomal enzymes for drug biotransformation (Brodie and Maickel 1962). Since then there have been several reports that have described oxygen- and NADPH-requiring hepatic enzyme systems, in addition to nitro group and azo linkage reductase systems in fish (Creaven et al. 1965, 1967; Ludke et al. 1972; Buhler and Rasmusson 1968a, 1968b; Adamson et al. 1965). In general, the activities of hepatic enzyme systems of fish appear lower than corresponding mammalian systems, possibly as a result of a deficiency of some component of the microsomal oxidase system (Buhler and Rasmusson 1968a). However, a recent report from this laboratory (Pedersen et al. 1974) has indicated that rainbow trout (*Salmo gairdneri*) possess aryl hydrocarbon hydroxylase (E.C. 1.14.14.2) activity that is severalfold greater than that observed in rat liver. The presence of such mixed function oxidase systems in fish indicates they may possess routes of detoxification similar to those demonstrated in mammals and insects (Culley and Ferguson 1969).

Acute doses of DDT have been found to alter levels of enzyme activity in the carp, *Cyprinus carpio* (Barry and Spicer 1971), and recent studies of rainbow trout, *S. gairdneri* (Pedersen et al. 1974), brown trout, *S. trutta*, and capelin, *Mallotus villosus* (Payne and Penrose 1975) have demonstrated inducibility of aryl hydrocarbon hydroxylase in the liver.

To date there has been little attention given to variation in drug metabolism due to strain dif-

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TABLE 1. Descriptions of rainbow trout (*Salmo gairdneri*) strains utilized in enzyme assays.

Strain	Origin	Average length (*cm*)	Average weight (g)	Age (*yr*)	Remarks
Spokane	Spokane, Wash.	19	85	<1	Hatchery reared in cool clean spring water
Mt. Whitney	California Fish and Game	17	65	<1	Hatchery reared in cool clean spring water
Chambers Creek	Chambers Creek, Wash.	21	106	2	Hatchery reared winter-run steelhead; pulpmill pollution at mouth of creek for 30 yr
Hagerman	Hagerman, Idaho	28	270	3	Hatchery reared in warm clean spring water
Donaldson	University of Washington, Seattle, Wash.	32	450	<1	Very fast growing strain; hatchery reared in lake water of varying temperature and degree of pollution
Chester Morse	Chester Morse Lake, Wash.	40	800	3–6	Natural population from a lake in pristine condition

ferences with a species of fish. It is possible that variation in the capacity to metabolize foreign compounds may appear during the development of a strain under different environmental conditions. It is the goal of the present study to determine whether or not differences exist in the rates of biotransformation of three environmental xenobiotics by hepatic systems of six strains of rainbow trout.

Materials and Methods

ANIMALS

Livers from six geographically and genetically distinct strains of rainbow trout were utilized (Table 1). All fish were maintained at the University of Washington College of Fisheries in freshwater troughs at temperatures ranging from 6.7 to 12.2 C under natural photoperiod. The water supply came directly from Lake Washington. All fish were fed trout pellets prepared at the University of Washington (Ramos-Henao 1970). The fish (except in those cases indicated) were acclimated to these conditions for at least 1 mo. Comparative experiments were performed between October and February.

TISSUE PREPARATION

Fish were killed by cervical dislocation and the livers immediately removed and placed on ice. Individual livers were homogenized in a teflon–glass homogenizer with two volumes of 1.15% KCl (isotonic). The homogenates were centrifuged at 10,000 × *g* for 20 min in an I.E.C. refrigerated centrifuge at 4 C. The resulting 10,000 × *g* supernatant fractions were utilized in the incubations. Where noted, some supernatant fractions utilized were frozen at −85 C for up to 2 wk prior to assay. Freezing in this fashion resulted in a decrease of 0–8% in enzyme activity.

ENZYME ASSAYS

The following enzymatic reactions (diagramed in Fig. 1) were studied in vitro: Aryl-4-monooxygenase (E.C. 1.14.14.1) by measurement of rates of conversion of aniline to *p*-aminophenol; nitro reductase by measurement of rates of formation of *p*-aminobenzoic acid from *p*-nitrobenzoic acid; and benzopyrene-3-monooxygenase (E.C. 1.14.14.2, also referred to as aryl hydrocarbon hydroxylase or AHH) by measurement of the formation of 3-hydroxybenzo[a] pyrene from the substrate benzo[a]pyrene.

a. Hydroxylation of aniline — The procedures

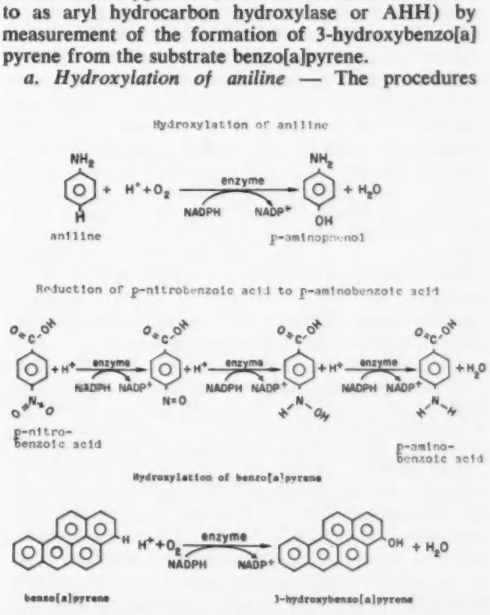

FIG. 1. Enzymatic reactions of hepatic systems measured in various strains of rainbow trout (*Salmo gairdneri*).

used follow closely those of Buhler and Rasmusson (1968a). Fresh fish enzyme preparations were incubated, in duplicate, in a Dubnoff metabolic shaking incubator at 25 C for 25 min. Typical reaction mixtures contained 0.6 ml enzyme (20 mg protein), 2.5 mg of neutralized aniline HCl (6.4×10^{-3} M final concentration), 0.27 mg NADPH (10^{-4} M final concentration) and sufficient 0.1 M potassium phosphate buffer, pH 7.4, to yield a final volume of 3.0 ml. Incubation preparations utilizing heat denatured enzyme were employed as blanks, and exhibited negligible activity. Incubation preparations containing 51.5 nmol of *p*-aminophenol without substrate were employed as standards. Incubations were carried out under a saturated oxygen gas phase.

Following the incubations, the preparations were extracted with 18 ml of ether containing 1 g NaCl. The organic phase was then extracted with 0.5 M $NaPO_4$ buffer (pH 12+) containing 0.8% phenol. This aqueous phase containing an indophenol blue complex was allowed to develop for 30 min, and the optical density was measured at 620 nm. Specific activities were expressed as nanomoles *p*-aminophenol formed per gram protein per minute.

b. Reduction of p-nitrobenzoic acid to p-aminobenzoic acid — The procedure used was a modification of that established by Buhler and Rasmusson (1968b). The enzyme preparations utilized were prepared in the same fashion as those for studies of aniline hydroxylation. Preparations were incubated in duplicate, at 25 C for 1 h under 100% nitrogen. Typical reaction mixtures contained 0.4 ml hepatic 10,000 × *g* supernatant, 0.2 mg of neutralized *p*-nitrobenzoic acid (6×10^{-4} M final concentration), 1.04 mg of NADPH (6×10^{-4} M final concentration), 0.5 mg of flavin mononucleotide (5×10^{-4} M final concentration), and sufficient 0.1 M potassium phosphate buffer pH 7.4 to make a final volume of 2.0 ml. Preparations utilizing enzyme boiled for 5 min were employed as blanks. Preparations containing 103 nmol of *p*-aminobenzoic acid but no substrate were employed as standards.

Nitro reductase activity was determined colorimetrically at 540 nm after trichloroacetic acid precipitation of protein, followed by the Bratton–Marshall reaction to yield a colored derivative, as described by Fouts and Brodie (1957). Specific activities were expressed as nanomoles *p*-aminobenzoic acid formed per gram protein per minute.

c. Hydroxylation of benzo[a]pyrene — The procedure used was a slight modification of that of Pedersen et al. (1974). Preparations were incubated in duplicate, for 10 min at 28.5 C, under a saturated oxygen gas phase. Typical reaction mixtures contained 1.0 ml of diluted (1:29 with 1.15% KCl) 10,000 × *g* supernatant fraction (which had been frozen) containing between 1.0 and 4.4 mg protein; 0.1 mg benzo[a]pyrene (10^{-4} M final concentration) dissolved in 0.02 ml acetone; 0.7 mg NADPH (2×10^{-4} M final concentration); and 0.1 M potassium phosphate buffer (pH 7.5) in a total volume of 4.0 ml. Supernatant fractions heated 5 min at 100 C were completely inactive, and preparations containing these were employed as blanks.

In preliminary experiments designed to examine inducibility in trout of hepatic aryl hydrocarbon hydroxylase and nitro reductase, fish were injected intraperitoneally 48 h prior to being killed, with varying amounts of 3-methylcholanthrene (3-MC) suspended in corn oil. Dosage ranged from 4 to 195 mg of 3-MC/kg body weight for the Donaldson strain and 75 mg of 3-MC/kg in other strains. Controls were treated with equal volumes of corn oil.

In all of the above assays, reactions were linear with time and over the ranges of protein concentrations utilized.

Spectral Analyses

In mammalian systems, cytochrome P-450 is thought to serve as the terminal oxidase for microsomal mixed function oxidases (Cooper et al. 1965). Chan et al. (1967) indicated the cytochrome P-450 content of rainbow trout liver was quite substantial. In addition, there was a considerable quantity of material absorbing at 420 nm. To determine whether or not there exists a relation between capacity for hepatic biotransformation of xenobiotics and the relative amount of cytochrome P-450, spectral analyses were performed on tissue preparations of both control and 3-MC pretreated groups of the strains that exhibited the highest and lowest activities of AHH.

Spectral analyses were performed with an Aminco-Chance (Model DW-2) recording spectrophotometer. CO-reduced minus CO-oxidized difference spectra of dilute hepatic 10,000 × *g* supernatant fractions of both control and 3-MC pretreated individuals from the Chambers Creek and Chester Morse strains were analyzed according to the method of Greim (1970). For each determination the 10,000 × *g* supernatant fractions of hepatic homogenates were diluted to a protein concentration of 2–3 mg/ml. A 2.5-ml aliquot was placed into each of two matching cuvettes of 1 cm path length. Carbon monoxide of 99.7% purity was bubbled through both the reference and sample cuvette for 60 s. The cuvettes were then placed in the spectrophotometer and the baseline was adjusted to a straight horizontal line. A few grains of sodium hydrosulfite were then added to the sample cuvette and the difference spectra were recorded. The machine was carefully calibrated using a holmium oxide filter as a standard.

In calculations of the concentrations of absorbing material at 450 nm, the extinction coefficient for cytochrome P-450 was used. The difference in absorbance (per milligrams protein) at 450 nm and 500 nm (the isosbestic point) was divided by the extinction coefficient of 100 mM^{-1} cm^{-1}. Values of the concentration of absorbing material at 450 nm were expressed in nmol/mg protein.

Protein Determinations

Protein concentrations for all of the above pro-

TABLE 2. In vitro hydroxylation of aniline to *p*-aminophenol by hepatic enzymes of various trout strains.

Strain	nmol product formed/g protein per min		
	Male	Female	Combined sexes
Chambers Creek	6.35±1.75 (10)[a]	9.80±2.52 (10)	8.07±2.75 (20)
Chester Morse	4.93±0.68 (5)	6.87±2.74 (7)	6.04±2.23 (12)
Donaldson	5.27±1.26 (9)	9.83±2.82 (4)	6.41±2.59 (13)
Hagerman	5.95±0.18 (2)	6.20±1.41 (8)	6.11±1.10 (10)
Spokane	9.56±3.31 (9)	6.90±1.28 (7)	8.23±2.72 (16)
Mt. Whitney	6.12±0.84 (9)	6.90±2.49 (9)	6.41±1.53 (18)

[a]Values indicated are means ± 1 SD. Numbers of determinations are indicated in parentheses.

cedures were determined according to the method of Lowry et al. (1951).

Statistical Procedures

Analysis of variance and Newman–Keuls procedure of the Studentized range statistic for unequal sample size were used to test differences between all pairs of means of specific activity of the various fish strains for a particular enzymatic reaction. Student's *t*-test was utilized in comparisons of differences between sexes and between treated and untreated fish. All statistical procedures used in the analysis of the results are described by Winer (1962).

Results

Hydroxylation of Aniline

Incubation of aniline with the 10,000 × *g* supernatant fraction of livers from various rainbow trout strains demonstrated that all of the six strains studied had the capacity to hydroxylate the substrate. The range of aniline hydroxylase activity for all individuals was 3.62–14.51 nmol product formed/g protein per min. The mean specific activity of all individuals combined (6.98 ± 2.38 nmol *p*-aminophenol formed/g protein per min) was slightly lower than activity of approximately 9.3 nmol product formed/g protein per min observed by Buhler and Rasmusson (1968a) who utilized rainbow trout stock from the Western Fish Nutrition Laboratory in Cook, Washington.

Females of the Chambers Creek and Donaldson strains exhibited significantly higher ($P < 0.05$) activity than males with respect to aniline hydroxylase (Table 2). There were no significant differences in specific activity between sexes of any other strain with respect to this enzymatic reaction. For comparative purposes, the mean specific activity of each strain, irrespective of sex, was used.

Highest mean aniline hydroxylase activity was observed in the Spokane strain, followed by Chambers Creek, Mt. Whitney, Donaldson, Hagerman, and Chester Morse strains (Table 2). There was no statistically significant difference between the mean specific activities of any two strains. However, analysis of variance indicated effects of strain ($F = 2.89$) were equal at the 0.01 level [$F_{.99}(5,83) = 3.25$] but contradicted the hypothesis of no strain differences at the 0.05 level [$F_{.95}(5,83) = 2.33$].

Nitro Reductase

Incubation of *p*-nitrobenzoic acid with the 10,000 × *g* supernatant fraction showed that all of the strains examined had the ability to reduce aromatic nitro groups. Specific activity for all individuals ranged from 0.018 to 0.400 nmol product formed/g protein per min. The average was 0.178 ± 0.019 nmol product formed/g protein per min. This compares to a value of 0.259 nmol product formed/g protein per min observed by Buhler and Rasmusson (1968b).

There was a slight increase in total mean nitro reductase specific activity observed in trout pretreated with 3-MC 48 h prior to being killed. However, it was not significant ($P > 0.05$), and observations from induction experiments were included with the controls when comparisons between strains were analyzed. No significant difference in metabolism of *p*-nitrobenzoic acid was observed between males or females of any strain. Therefore, mean specific activities of all fish within a strain were used in making comparisons between strains (Table 3). As with the comparisons of aniline hydroxylase, the three strains which exhibited the highest specific activities were Spokane, Chambers Creek, and Mt. Whitney. Following these were the Hagerman, Donaldson,

TABLE 3. Metabolism of *p*-nitrobenzoic acid to *p*-aminobenzoic acid by hepatic enzyme systems of various strains of rainbow trout.

Strain	Treatment	nmol product formed/g protein per min				Range statistic[a]
		Male	Female	Combined sexes	Combined treatments	
Spokane	Control	0.36±0.06 (9)[b]	0.27±0.08 (7)	0.32±0.08 (16)	0.31±0.08 (22)	]
	3-MC	0.24±0.06 (2)	0.32±0.08 (4)	0.29±0.08 (6)		
Chambers Creek	Control	0.18±0.03 (10)	0.22±0.07 (10)	0.20±0.05 (20)	0.20±0.05 (20)	]
Mt. Whitney	Control	0.19±0.02 (9)	0.16±0.03 (9)	0.17±0.04 (18)	0.17±0.04 (26)	]
	3-MC	0.23±0.04 (3)	0.13±0.07 (5)	0.18±0.06 (8)		
Hagerman	Control	0.26±0.02 (2)	0.15±0.08 (8)	0.17±0.09 (10)	0.17±0.09 (10)	]
Donaldson	Control	0.11±0.04 (10)	0.11±0.06 (10)	0.11±0.04 (20)	0.11±0.04 (20)	]
Chester Morse	Control	0.06±0.05 (5)	0.10±0.06 (7)	0.08±0.05 (12)	0.09±0.05 (17)	]
	3-MC	0.11 (1)	0.12±0.02 (4)	0.12±0.02 (5)		

[a]Strains bracketed did not differ significantly. Strains not included in the same bracket did differ. Significance level chosen was $P < 0.01$.

[b]Values indicated are ± 1 SD. Number of determinations are indicated in parentheses. The treatment 3-MC indicates that the animals were injected i.p. 48 h prior to being killed with approximately 75 mg of 3-methylcholanthrene/kg body weight. Controls were injected with corn oil.

TABLE 4. Hydroxylation of benzo[a]pyrene to 3-OH-benzo[a]pyrene by hepatic enzymes of various strains of rainbow trout.

Strain	μmol 3-hydroxybenzo[a]pyrene formed/g protein per min			Range statistic[a]
	Male	Female	Combined sexes	
Chambers Creek	2.29±2.66 (12)[b]	2.26±3.07 (13)	2.28±2.82 (25)	]
Spokane	1.76±0.23 (9)	1.35±0.39 (7)	1.51±0.38 (16)	]]
Mt. Whitney	1.35±0.49 (9)	1.33±0.46 (9)	1.34±0.43 (18)	]
Hagerman	0.44±0.11 (2)	0.70±0.38 (8)	0.65±0.35 (10)	]
Donaldson	0.57±0.20 (56)	0.50±0.15 (36)	0.55±0.18 (92)	]
Chester Morse	0.04±0.04 (5)	0.05±0.04 (7)	0.04±0.04 (12)	]

[a]Strains bracketed do not differ statistically. Strains not bracketed do differ. Significance level chosen was $P < 0.01$.

[b]Values indicated are mean specific activities ± 1 SD. Numbers of determinations are indicated in parentheses.

and Chester Morse strains. An analysis of variance test gave an F value of 29.7 with df (5,109), which signifies ($P < 0.01$) there are differences between strains in relation to mean specific activity. Results of the Studentized range statistic test indicated the Spokane strain exhibited significantly higher hepatic nitro reductase activity than any of the other strains.

ARYL HYDROCARBON HYDROXYLASE

Hepatic AHH was present in at least some members of all strains studied (Table 4). There was no significant difference observed between males and females in their capacity to hydroxylate benzo[a]pyrene. For this reason the mean specific activity of all fish within a strain was used for comparisons between strains in relation to hepatic AHH.

The highest variability within a strain was observed in the fish from Chambers Creek. The range of hepatic AHH specific activity was from 0.08 to 9.18 μmol product formed/g protein per min. This was the only strain that contained individuals that had basal specific activities greater than 2.00, and it also had the highest mean specific activity. Following the Chambers Creek strain in decreasing mean specific activity were the Spokane, Mt. Whitney, Hagerman, Donaldson, and Chester Morse strains. An analysis of variance was conducted to test if there existed differences between means. An F value of 12.90 with df (5,167) was obtained. This is significant ($P < 0.01$), and indicates there are differences between mean specific activities of the various strains. Results of the Studentized range statistic are also shown in Table 4.

INDUCTION OF ARYL HYDROCARBON HYDROXYLASE

In preliminary induction experiments it was observed that trout hepatic AHH activity was increased when 3-MC was injected in dosages as low as 4 mg/kg body weight. The vehicle, corn oil, was found to have no effect on enzymatic activity. Several doses were administered to members of the Donaldson strain, but the responses were highly variable between individuals at each dose. Although there was a slight positive correlation between dosage of the inducing compound and increase in activity of hepatic AHH, it was not possible to determine a workable dose–response relation with these data.

Comparative experiments of induction indicated that at least some members in each strain had increased capability to metabolize benzo[a]pyrene after being pretreated with 3-MC (Table 5). When compared to controls, no statistically significant ($P > 0.05$) increase in the quantity of protein per gram liver was observed in 3-MC pretreated fish. There was a significant increase in mean specific activity of each strain when members were pretreated with 3-MC. There was no significant difference in the magnitude of induction with respect to sex; therefore, mean specific activities of all 3-MC pretreated individuals within a strain were used for comparisons. The greatest magnitude of induction was observed in the Chester Morse strain, where mean specific activity of 3-MC pretreated individuals was 47.8 times higher than controls. The ratios of means of induced to control specific activities for the remaining strains were as follows: Spokane — 3.6:1, Chambers Creek — 3.01:1, Mt. Whitney — 2.51:1, and Donaldson — 2.3:1. Induction was not examined in the Hagerman strain due to the unavailability of fish. Analysis of variance of all 3-MC pretreated individuals yielded an F value of 15.97 with df (4,45), and indicated that mean specific activity for induced hepatic AHH activities were significantly ($P < 0.01$) different

TABLE 5. Hydroxylation of benzo[a]pyrene to 3-OH-benzo[a]pyrene by induced[a] hepatic enzymes of various strains of rainbow trout.

Strain	nmol product formed/g protein per min			Range statistic[b]
	Male	Female	Combined sexes	
Chambers Creek	6.50±4.71 (5)[c]	6.96±3.81 (5)	6.73±4.05 (10)	]
Spokane	5.14±1.80 (2)	5.52±1.18 (4)	5.39±1.24 (6)	]
Mt. Whitney	3.01±0.58 (3)	3.57±0.87 (5)	3.36±0.78 (8)	]
Chester Morse	2.11 (1)	1.86±0.85 (4)	1.91±0.74 (5)	]
Donaldson	1.40±0.74 (11)	1.12±0.43 (10)	1.27±0.61 (21)	]

[a]Animals were injected i.p. 48 h prior to being killed with approximately 75 mg of 3-methylchloanthrene/kg body weight.

[b]Strains bracketed do not differ statistically. Strains not bracketed do differ. Significance level chosen was $P < 0.01$. Examination of the Hagerman strain was not performed due to unavailability of fish.

[c]Values indicated are mean specific activities ± 1 SD. Numbers of determinations are indicated in parentheses.

among the strains tested. The test for differences in pairs of means of induced specific activities of trout indicated (Table 5) that the means of the Chambers Creek, Spokane, and Mt. Whitney strains were not different. However, the mean induced specific activities for benzo[a]pyrene metabolism did differ between the Chambers Creek and the Mt. Whitney strains at a significance of $P < 0.05$.

ENZYME KINETICS

As shown from Lineweaver–Burk plots in Fig. 2 and 3, the apparent K_m and V_m for the 3-hydroxylation of benzo[a]pyrene does differ between strains and between control and 3-MC pretreated groups within strains. Specific activities were determined on pooled individuals within groups of 3-MC pretreated and control fish of the two strains. For the Chambers Creek strain, the theoretical V_m was determined to be approximately 6.01 and 10.01 μmol product formed/g protein per min for controls and 3-MC pretreated fish, respectively. Corresponding values for the Spokane strain were 1.72 and 4.81 μmol product formed/g protein per min. The apparent K_m for the enzymatic reaction in the Chambers Creek strain was approximately 2.8×10^{-6} M and 3.09×10^{-6} M, respectively, for control and 3-MC pretreated fish. Corresponding values for the Spokane strain were approximately 5.15×10^{-6} M and 1.44×10^{-5} M. Values were determined by linear regression. The apparent K_m for 3-MC induced AHH in rats is approximately 2.5×10^{-5} M (Fouts 1973), which suggests that the fish enzyme may have a slightly higher affinity for the substrate than the enzyme in rats.

The slopes of lines for 3-MC pretreated and control fish of the Spokane strain are not significantly different. Also, from Fig. 2 it appears that the lines representing 3-MC pretreated and control fish of the Chambers Creek strain intersect the abscissa in approximately the same place. However, the points of intersection are significantly different due to extremely low variance about the lines of both plots.

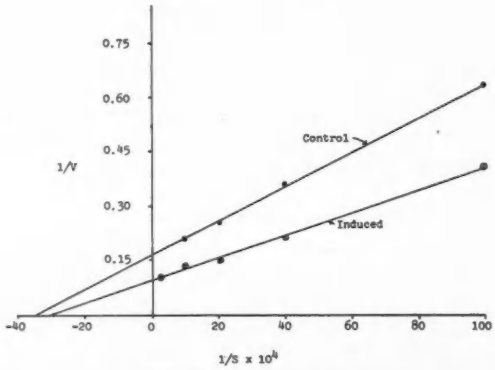

FIG. 2. Double reciprocal plot of benzo[a]pyrene concentration against rate of hydroxylation by hepatic 10,000 × *g* supernatant fractions of 3-MC pretreated and control members of the Chambers Creek strain. Velocities were expressed as micromoles product formed per gram protein per minute and substrate concentrations were in moles liter^{-1}. Incubation conditions were the same as those reported in Table 1.

SPECTRAL ANALYSES

CO-reduced minus CO-oxidized difference spectra of hepatic 10,000 × *g* supernatant fractions from the trout strains that exhibited the highest and lowest AHH activities were determined and the results are illustrated in Fig. 4.

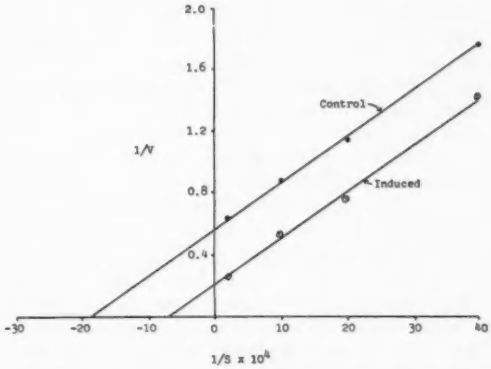

FIG. 3. Double reciprocal plot of benzo[a]pyrene concentration against rate of hydroxylation by hepatic 10,000 × *g* supernatant fractions of 3-MC pretreated and control members of the Spokane strain. Velocities were expressed as micromoles product formed per gram protein per minute and substrate concentrations were in moles liter^{-1}. Incubation conditions were the same as those reported in Table 1.

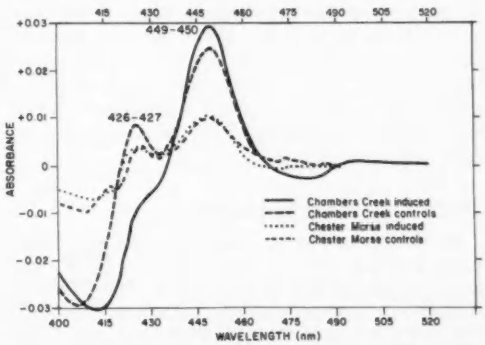

FIG. 4. CO-reduced minus CO-oxidized difference spectra of dilute hepatic 10,000 × *g* supernatant fractions from 3-MC pretreated and control groups of the Chambers Creek and Chester Morse strains. Protein concentrations were approximately 2.0 mg/ml.

It revealed that there are variable amounts of hemoproteins, which, when complexed with CO, yielded absorption maxima at 426–429 nm and 449–450 nm.

Concentrations of CO-binding pigment at 450 nm for the various tissue preparations were calculated. The Chambers Creek strain exhibited a concentration of 0.120 nmol/mg protein in the 10,000 × *g* supernatant compared to 0.137 nmol/mg protein in that of the treated group. However, the Chester Morse strain exhibited a concentration of 0.050–0.052 nmol/mg protein in liver preparations of both control and treated groups. The concentration of CO-binding pigment of the control group of the Chester Morse strain was only 42% of the corresponding group of the Chambers Creek strain. A similar relation existed between the 3-MC pretreated groups of the two strains. A slight increase in content of CO-binding pigment was observed in groups pretreated with 3-MC over control groups. However, no direct relation could be established between relative capacity for hepatic biotransformation and concentration of CO-binding pigment. For example, the hepatic AHH activity of the Chester Morse strain increased 4675% upon pretreatment with 3-MC and the concentration of CO-binding pigment at 450 nm increased only 4% with the same treatment.

Administration of 3-MC to trout at doses producing increased hepatic AHH activity does not produce a shift of the P-450-CO difference spectra from 450 to 448 nm, as is the case in studies of the rat (Fouts 1973).

Discussion

Experiments in vitro by Brodie and Maickel (1962) suggested that hepatic microsomal enzymes of fish could not catalyze dealkylation, hydroxylation, or deamination of xenobiotics. However, more recent studies, reviewed by Adamson (1967), and the present investigation have demonstrated that hepatic enzyme systems of fish carry out a number of mixed function oxidase and NADPH-dependent reduction reactions.

Much variation in hepatic AHH activity was observed between strains and individuals within some strains. As demonstrated from the results of spectral analyses and kinetics studies, it may be that substantial differences in the characteristics of the hepatic AHH systems exist, not only between various strains of rainbow trout, but between fish and mammalian systems as well. However, caution should be employed in interpreting the data because unknown factors such as age, size, and rearing conditions varied between strains. Many of the characteristics observed were considerably different from those of mammalian systems. Among these were temperature optima (Pedersen et al. 1974), the apparent affinity of the enzyme for substrate and cofactors, and spectral properties.

Several possibilities exist regarding the mechanism of expression of trout hepatic AHH in both 3-MC pretreated and control members of the various strains. Although there was little supporting evidence, more AHH-degrading enzymes may be present in individuals with low activity. Individuals with high activity may have mutant genes

that cause the production of more AHH, or they may exhibit gene redundancy providing more sites for induction and enzyme production. Observed variation may also be due to different modes of enzyme regulation.

If the observed difference in specific activity of AHH between 3-MC pretreated and control animals is due to different enzyme structures, one might expect a difference in the apparent Michaelis–Menten constant (K_m). In at least one strain (Chambers Creek) there was no large difference in K_m between 3-MC pretreated and control groups of pooled individuals. However, in the 3-MC pretreated group of the Spokane strain, the induced enzyme exhibited a lower affinity for substrate than the enzyme of the control group.

Due to the extensive variation observed in this study it is possible that some or all of the mechanisms mentioned previously may be interacting. This indicates the need for extensive additional research on the enzymology of individuals of each strain before any conclusions can be made about the species as a whole. It would be best to utilize an inbred line of rainbow trout (similar to inbred lines of rodents) to minimize genetic variation during the characterization of various enzyme systems in this species. However, such lines of trout are difficult to develop and therefore are very rare.

Further investigations are necessary in order to better understand the sensitivity of trout AHH to this enzyme stimulating compound. These should include examination of effects of various methods of administration of 3-MC, threshold dosage necessary to observe stimulation of AHH, and effects of chronic exposure of trout to various amounts of 3-MC.

For the three enzyme systems studied, a pattern was observed in the way the individual strains ranked with respect to their capacity for metabolism. The Spokane and Chambers Creek strains ranked highest. The Mt. Whitney strain was always at least third highest, the Hagerman and Donaldson strains were usually second lowest, and the Chester Morse strain exhibited the lowest activity, except in the absolute amount of induced hepatic AHH activity. The Chester Morse strain, however, exhibited the greatest capacity for induction of the AHH enzyme, more than 15 times the magnitude of induction observed in other strains. This may suggest that effects of the environment may have a significant influence on the expression of genes which regulate biotransformation by this enzyme.

It is possible that the pulpmill effluent near Chambers Creek may have been a factor that selected for fish with high capacity for hepatic biotransformation of polluting compounds, because fish passed through this effluent as outmigrants and returning spawners. The Spokane strain, in contrast, has been developed in clean spring water near Spokane, Washington. However, in this agricultural area the strain may have been exposed to some unknown pollutant. In addition, the possibility cannot be ruled out that the predecessor of the Spokane strain in Massachusetts may have developed under conditions of pollution. The pristine environment of Lake Chester Morse may have been a determinant in the exhibition of reduced initial capacity for drug metabolism in the strain from that lake. No measurable correlation between environment and the development of the capacity for drug biotransformation can be made from the results of this study.

The results of the present study emphasize the fact that use of undesignated strains may make comparisons of data about drug disposition from one laboratory to another even more difficult than it is now. However, some caution must be exercised in interpreting the results of the present study beyond the observation that there may exist differences in some hepatic enzymes between geographically and genetically distinct strains of rainbow trout. Interpolating in vitro observations to in vivo situations is often highly speculative, but offers a starting point for further investigations.

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Thermal Tolerance of Stored *Fundulus heteroclitus* Gametes: Fertilizability and Survival of Embryos

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KUCHNOW, K. P., AND R. S. FOSTER IV. 1976. Thermal tolerance of stored *Fundulus heteroclitus* gametes: fertilizability and survival of embryos. J. Fish. Res. Board Can. 33: 676–680.

Fundulus heteroclitus ova were tested for fertility after storage intervals of 6, 12, 24, 48, 72, and 96 h over a temperature range of 0–30 C (2-degree increments). Sperm were tested after storage intervals of 2, 4, 6, 12, 15, and 24 h over the range of 0–20 C. Ova retained fertility best at 10–14 C, with fertilization rates of 93, 80, and 46% after 24, 48, and 72 h, respectively. Sperm retained fertility best at 0–4 C, with fertilization rates of 85, 59, and 25% after 2, 4, and 6 h, respectively. The shorter time that sperm retained fertility, compared to the ova, was attributed to sperm activity, since all gametes tested were obtained by stripping, which initiates motility in *F. heteroclitus* sperm.

Variation in mortality in stored ova could be attributed to temperature differences. No relation between sperm storage temperatures and embryo mortality was found.

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Nous avons vérifié la fertilité d'ovules de *Fundulus heteroclitus* après entreposage de 6, 12, 24, 48, 72 et 96 h à des températures s'étalant de 0 à 30 C (augmentations de 2 degrés). Le sperme a été vérifié après intervalles d'entreposage de 2, 4, 6, 12, 15 et 24 h sur une gamme de 0–20 C. Les ovules conservent le mieux leur fertilité à 10–14 C, avec taux de fécondation de 93, 80 et 46% après 24, 48 et 72 h respectivement. Le sperme conserve le mieux sa fertilité à 0–4 C, avec taux de fécondation de 85, 59 et 25% après 2, 4 et 6 h respectivement. Nous attribuons à l'activité des spermatozoïdes la plus courte période de fertilité du sperme, comparativement aux ovules, car toutes les gamètes utilisées lors des essais ont été obtenues par pression des flancs, ce qui déclenche la motilité des spermatozoïdes de *F. heteroclitus*.

La variation dans la mortalité d'ovules entreposés peut être attribuée à des différences de température. Nous n'avons pas trouvé de relation entre les températures d'entreposage du sperme et la mortalité des embryons.

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THE effect of storage temperature on the fertilizing capacity of teleost gametes has been examined for a number of species. The studies have concentrated primarily on commercially valuable fishes such as the Salmonidae (Table 3). Most tests were performed on gametes stored either at one temperature, or over a variable but narrow range. Tests on nonsalmonid gametes have also been performed over a limited temperature range, and in many the gamete fertility was not tested (reviewed by Ginsberg 1972).

The present study, using *Fundulus heteroclitus* gametes, was initiated to examine the usefulness of temperature as a means of preserving the fertilizability of teleost gametes for extended time periods required for proposed National Aeronautics and Space Administration (NASA) studies on the effects of space environment on vertebrate embryogenesis. The study examines the effects of a wide range of storage temperature on gamete fertility and on survival and development of the embryos.

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Materials and Methods

Specimens of *Fundulus heteroclitus* were obtained from a breeding colony maintained at the NASA laboratories in Houston, Texas. The original stock was collected at Beaufort, North Carolina. The adults were maintained at a fairly constant temperature (20 ± 2 C) in the laboratory where the physical environment (salinity 20‰, 16 h light, 8 h dark) was adjusted to stimulate continuous gamete production (Boyd and Simmonds 1974). In the Beaufort, North Carolina, area the breeding season normally runs from June to August (Brummett 1966) in these estuarine waters at temperatures in excess of 20 C.

Gametes for testing were obtained by stripping rather than killing the animals. Sperm were collected from the spermatic aperture in a Pasteur pipette provided with a suction bulb. Small quantities

were harvested and pooled. Fecal matter, urine, mucous, and other sources of contamination were occluded from the sperm sample.

The storage temperatures to be tested were maintained in a Lauda–Binkman K-2/R circulating water bath. Ova held at single temperatures ranging from 0 to 30 C at 2 degree increments were tested for fertilizability at intervals of 6, 12, 24, 48, 72, and 96 h. Sperm held at single temperatures from 0 to 20 C at 2 degree increments were tested at intervals of 2, 4, 6, 12, 15, and 24 h.

Freshly stripped ova were placed in small plastic capsules (Beem, Inc.) capable of holding approximately 150 ova. Capsules were filled to the top so that a minimal air space remained after capping. No supportive media were added other than the ovarian fluid which accompanied the ova when stripped. The capsules were capped and placed in the water bath set at the test temperature. At appropriate time intervals the capsules were removed from the bath, the ova poured out, allowed to reach ambient temperature (22–25 C) and tested for fertility by applying fresh sperm. A similar technique was used to test the stored sperm. Temperature-treated sperm were tested with fresh ova. Each test was replicated at least 4 times, making a total of 500–600 ova per test-temperature.

Percent fertility was based on examination of all exposed ova and was determined as the number reaching the two to four cell stage of development divided by the total number of ova exposed to sperm. The percent fertility of the four replicates at each temperature were averaged for graphic representation. Mortality and abnormalities were monitored until hatching. No separation was made between mortality and abnormality since the latter eventually led to mortality. Controls, involving fresh sperm and ova were run during the same period as the tests of stored gametes, at random intervals; these consistently gave fertilization rates 90% or better, with less than 5% mortality, and hatching occurred within 14–21 days.

Results

Data on the effect of temperature and storage time on the fertilizability of *F. heteroclitus* ova (Fig. 1) show that at the shortest time intervals, 6 or 12 h, the ova demonstrated high fertilization rates over a broad range of temperature (6–20 C). Fertilization rates fell off rapidly at both the upper and lower extremes of temperature. At the 24-, 48-, and 72-h test periods, the best fertilization rate occurred in the range 10–14 C. In isolated tests at 12 C, after 120 and 168 h, fertilization rate was 42 and 15%, respectively. After storage for these periods, development of the embryos was slower than normal and the majority of eggs failed to hatch.

Storage temperature of ova prior to fertilization is related to the survival of the resulting embryos

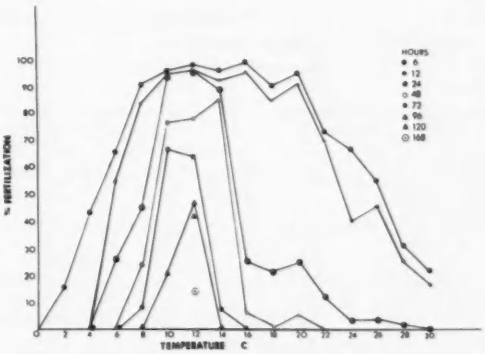

FIG. 1. Percentages of *Fundulus heteroclitus* ova fertilized (by fresh sperm) after storage for various times at different temperatures.

(Table 1). Below 12 C and above 20 C, mortality was relatively high, and most deaths occurred within 2 days of fertilization. In those cases, early development was marked by irregular cleavage, indicative of abnormality and early death of embryos. At 12, 14, and 16 C mortality was generally lower, but as exposure time increased, there was a tendency toward a higher proportion of abnormal development and premature death.

Stored sperm showed a greater tolerance for lower temperatures (Fig. 2). Fertilizability was greatest at temperatures below 6 C. After 2 and 4 h of storage, fertilization occurred at all storage temperatures tested, but after 6 h only sperm kept at 8 C and lower were capable of fertilization. At temperatures and time intervals where low fertilization rates occurred, microscopic examination revealed minimal sperm motility.

Embryo mortality associated with stored sperm failed to exhibit any discernible pattern (Table 2). Most mortalities were within the 5% limit established for controls, and were low relative to those exhibited by the stored ova. At the only time–temperature combinations that the tested ova and sperm both showed fertilization, 6 h at 4, 6, and 8 C, mortality rates for the ova were 87.2, 65.0, and 20.4%, while those for the sperm were 0, 0, and 3.7%, respectively. Thus, while sperm are more susceptible to temperature, in terms of length of time that they retain fertilizability, than are ova, the sperm, when subjected to temperature stress, retained the ability to initiate normal embryo development better than did ova.

Discussion

The results indicate that within the 0–30 C temperature range tested, there are certain optimal temperatures for retention of fertilizing capacity.

TABLE 1. Percentage mortality of embryos from ova stored at different temperatures.

Temp (C)	Exposure time (h)					
	6	12	24	48	72	96
0	—[b]	—	—	—	—	—
2	—	—	—	—	—	—
4	87.2[a]	—	—	—	—	—
6	65.0	90.0	56.0	—	—	—
8	20.4	42.7	77.5	71.4	87.0	—
10	12.0	23.0	19.6	50.4	63.0	74.0
12	10.0	7.3	8.0	37.7	22.8	50.0
14	6.8	4.1	14.0	22.0	22.0	—
16	5.0	3.0	26.6	42.8	—	—
18	14.8	12.0	7.6	0	—	—
20	0	2.0	57.9	40.0	—	—
22	8.3	11.1	46.1	—	—	—
24	37.5	44.2	100.0	—	—	—
26	60.0	54.5	77.0	—	—	—
28	10.3	38.0	65.0	—	—	—
30	33.3	50.0	—	—	—	—

[a]Each value includes both mortalities and abnormalities.
[b]Fertilization did not occur.

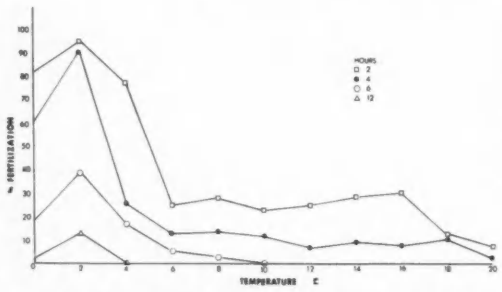

FIG. 2. Percentages of fresh *F. heteroclitus* ova fertilized by sperm stored for various times at different temperatures.

The stored ova were able to retain fertilizability for a considerable length of time which was temperature dependent; temperatures below 10 C and above 14 C shortened the period of fertilizability, and the range 10–14 C prolonged fertilizability. Optimum temperatures for preservation were 6–10 C below that of water in the adult tanks. These observations are applicable only to "dry" preserved ova and would not refer to the natural spawning conditions in which the ova are shed into water prior to fertilization. Survival time under these conditions would probably be shorter. Newman (1905) reported that *F. heteroclitus* ova would not be fertilized if they were exposed to sea water for more than 1 h, while Gee (1916) and Kagan (1935) reported fertilizable periods of only 20–30 min or 15–20 min, respectively.

TABLE 2. Percentage mortality of embryos from sperm stored at different temperatures.

Temp (C)	Exposure time (h)			
	2	4	6	12
0	4.0[a]	0	6.3	0
2	0	8.0	0	12.0
4	9.3	5.3	0	—[b]
6	0	5.3	0	—
8	10.3	0	3.7	—
10	2.3	0	—	—
12	4.1	9.7	—	—
14	0	10.0	—	—
16	0	0	—	—
18	0	7.7	—	—
20	6.3	3.2	—	—

[a]Each value includes both mortalities and abnormalities.
[b]Fertilization did not occur.

The duration of fertility of *Fundulus* ova is similar to that obtained for a number of species of salmon and trout (Table 3). Most of the salmonid tests were run over a very narrow temperature range and there is no way of knowing if the reported fertility or viability values are optimal. In some of these tests the gametes were stored at ambient spawning stream temperatures of 8–10 C (Foerster 1965; Withler and Morley 1968). Duration of fertility for *Fundulus* ova was optimal at temperatures below the ambient holding tank temperature.

The effect of temperature on stored sperm was different from that on ova with respect to tem-

TABLE 3. Summary of teleost sperm and ova fertility tests.

Species	Temp of preservation (C)	% Fertility of Sperm (S) or Ova (O)	Reference
A. Salmonidae			
Salmo fario	2.2–3.3	good — 4 days, poor — 5 days, none — 6 days (S)	Butcher (1944)
S. gairdneri	10	7 days — 58.5% (S) 8 days — 15.6% (S)	Nomura (1964)
S. gairdnerii gairdnerii	6.1–7.0	fertility not reported 2.5 h — 97% hatch mortality (S) 5 h — 99.7% hatch mortality (S)	Smith and Quistorff (1943)
S. ischehan	0–40	duration of motility 120 s — 2 C (S) 10 s — 35 C (S)	Turdakov (1971)
Oncorhynchus keta	2.5–5.8	90% — 3 days (S) (O) 35% — 8 days (O) trace — 11 days (S)	Barrett (1951)
" "	6	90% — 20 hr (S) (O) trace — 72 h (S) (O)	Poon and Johnson (1970)
O. nerka	2–8	74% — 4 days (O), 4.4% — 3 days (S)	Foerster (1965)
" "	8.4–8.5	50% — 22 h (O), 50% — 30 h (S)	Withler and Humphreys (1967)
" "	9.9	> 90% — 22 h, ~7% — 94 h (O) > 90% — 46 h, ~15% — 70 h (S)	Withler and Morley (1968)
" "	2.9	> 90% — 70 h, ~12% — 333 h (O) > 90% — 94 h, ~20% — 220 h (S)	Withler and Morley (1968)
O. gorbuscha	6	90% — 20 h (O) (S), trace — 72 h (O) (S)	Poon and Johnson (1970)
" "	8–9	> 90% — 33 h (S)	Withler and Humphreys (1967)
" "	8.5	> 90% — 8 h, ~25% — 94 h (O) > 90% — 46 h, ~30% — 94 h (S)	Withler and Morley (1968)
" "	3.2	> 85% — 46 h, ~15% — 144 h (O) > 90% — 46 h, ~10% — 190 h (S)	
O. tschawytscha	6.1–7.0	2.5 h — fertility not reported — 9.5% hatch mortality (S) 5 h — fertility not reported — 45.9% hatch mortality (S)	Smith and Quistorff (1943)
Salvelinus fontinalis	2	24% — 24 h (S)	Plosila et al. (1972)
B. Non Salmonidae			
Gillichthys mirabilis	2–4	fertility untested (S); motility after 2 wk	Weisel (1948)
Clupea harengus	−79	ova unviable 85% — 7 mo (S)	Blaxter (1953)
Cyprinus carpio and *Carassius auratus*	3–5	fertility not tested; motility after 30 days	Sneed and Clemens (1956)
	−73	fertility not tested; motility after 60 days	
Gadus morhua	−196	36 ± 12% — 60 days (S)	Mounib et al. (1968)
Mugil capito	10	fertility not tested; motility after 34 h (S)	Hines and Yashuov (1971)
Coregonus clupeoides	4	50% — 3 days (O) (S)	Maitland (1967)
Diptychus dybowskii	0–40	fertility not tested > 160 s motility — 5 C (S) ~10 s motility — 40 C (S)	Turdakov (1971)
Leuciscus bergi	0–40	5 C — 220 s motility — 95% (S) 30 C — 20 s motility — 30% (S)	Turdakov (1971)

perature sensitivity. The highest sperm fertility occurred in the range 0–4 C. At temperatures higher than 6 C, retention of fertility was markedly decreased. These times are shorter than storage times reported for salmonids and other species (Table 3), although the lower temperatures for best preservation are consistent with other teleost species data. The small quantities of sperm tested and collection by stripping could account for the short effective storage times.

Fundulus sperm retained fertility for a much shorter time than did the ova. The difference between sperm and ova may be attributed to the greater activity of the sperm. Ginsburg (1972) reported that the sperm of oviparous teleosts are inactive in coelomic fluid, but become active when diluted with sea water. Microscopic examination of *F. heteroclitus* sperm revealed that immediately upon ejaculation and exposure to air or water they become intensely active. This activity is relatively short and after several minutes all activity ceases. Gee (1916) reported that in sea water the sperm of *Fundulus* remain alive for only 8–14 min. Once motility ceases, fertilization will not occur. In the present study, because there was a time lapse of close to 2 min from collection and pooling of the sperm to introduction into the water bath, the activity could have been sufficient to seriously deplete energy reserves and affect subsequent motility and fertility. Preliminary studies (unpublished data) using excised, intact testes, gave up to 90% fertility after 72 h storage at 2 C. These data suggest that as long as motility is not initiated, sperm will retain their fertility longer.

Acknowledgments

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Distribution of Sound Scattering Layers Caused by Euphausiids and Their Relationship to Chlorophyll *a* Concentrations in the Gulf of St. Lawrence Estuary[1]

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SAMEOTO, D. D. 1976. Distribution of sound scattering layers caused by euphausiids and their relationship to chlorophyll *a* concentrations in the Gulf of St. Lawrence estuary. J. Fish. Res. Board Can. 33: 681–687.

A 3-yr study in the Gulf of St. Lawrence estuary using a 120-kHz sounder has shown the existence of a large population of euphausiids consisting of the species *Thysanöessa raschii*, *T. inermis*, and *Meganyctiphanes norvegica*. The sound scattering produced by these animals varied in intensity as the density and biomass m^{-3} of the animals. Chlorophyll *a* concentrations measured within 5 m of the surface at the same time as the sound scattering layers showed a significant correlation between the numbers of euphausiids m^{-3} and the concentration of chlorophyll *a* m^{-3}.

SAMEOTO, D. D. 1976. Distribution of sound scattering layers caused by euphausiids and their relationship to chlorophyll *a* concentrations in the Gulf of St. Lawrence estuary. J. Fish. Res. Board Can. 33: 681–687.

Une étude de 3 a. effectuée dans l'estuaire du golfe Saint-Laurent à l'aide d'un sondeur de 120 kHz a démontré l'existence d'une grande population d'euphausides constituée des espèces *Thysanöessa raschii*, *T. inermis* et *Meganyctiphanes norvegica*. La dispersion du son causée par ces animaux varie en intensité en fonction de la densité et de la biomasse m^{-3} des organismes. Les concentrations de chlorophylle *a* mesurées dans les 5 m superficiels en même temps que les couches dispersantes montrent une corrélation significative entre le nombre d'euphausides m^{-3} et la concentration de chlorophylle *a* m^{-3}.

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SONAR has been used to detect sound scattering layers caused by marine organisms for many decades. Moore (1950) and Boden (1950) suggested that euphausiids may be important components of the deep scattering layers (DSL). Kinzer (1970) found *Meganyctiphanes norvegica* the dominant species in the upper part of the DSL recorded at 30 kHz. All these studies found little relationship between the strength of the scattering layer and the biomass and distribution of the zooplankton in the layers. This was probably the result of the low frequency used. Bary and Pieper (1970) using a 197-kHz sounder found a relationship between sound scattering and the presence of high densities of euphausiids. Beamish (1971) made in situ measurements of the scattering of *Euphausia pacifica* using a 102-kHz sounder. McNaught (1968) gave a relationship between the diameter of spherical particle and the maximum back scattering strength for different frequencies. From the above data, 120 kHz was thought to be the optimal frequency for the study of euphausiids in the DSL. Berkes (1973) showed the area of major concentration of the three commonest species, *Thysanöessa raschii*, *T. inermis*, and *Meganyctiphanes norvegica* was between the Saguenay River and the eastern end of Anticosti Island. Therefore a number of surveys were made in this area to map the distribution of the euphausiids using high frequency sonar. In addition, chlorophyll *a* was measured in the region at the same time to determine if a relationship existed between the euphausiids and areas of high chlorophyll *a* concentration.

Methods

AREA SURVEYED AND ACOUSTIC EQUIPMENT

All surveys were conducted in the upper regions of the Gulf of St. Lawrence between the eastern tip of Anticosti Island and the Saguenay River.

A Simrad Scientific Sounder EK-R with a frequency of 120 kHz, a pulse length of 0.3 ms, and output of 0.8 kW was used. The transducer was mounted in a Braincon type 317 V-Fin and towed

[1]Bedford Institute of Oceanography Contribution.

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at a depth of 5 m with a Braincon type 317 hair faired cable. The transducer was towed over predetermined courses at a speed of 6 knots. The controls of the sounder for all surveys were set as follows:

Transducer:	1 (narrow beam, 3 kHz)
Range:	0–125 m or 0–250 m
Line density:	38 lines/cm
TVG and gain:	20 log R, 0 db
Recorder mode:	Norm
Bandwidth and pulse:	10 kHz, 0.3 ms
Duration:	2
Transmitter:	1/1 (0.8 kW output pulse power)
Recorder gain:	8
Discriminator:	4

The range was set at 0–250 m when the scattering layers sank below 125 m. A second transducer, towed at a depth of 50 m, had the sound beam pointed upward to detect sound scattering layers from 50 m to the surface. The frequency and setting of this sounder was the same as mentioned above. This transducer was used only during 1974.

During each of the surveys the temperature profile was regularly taken with a bathythermograph to ensure that the sound scattering layers being observed were not the result of water density differences. At no time did temperature differences produce back scattering strong enough to be confused with that from the animals.

Classification of Scattering Layers

The scattering layers were subjectively classified into heavy, medium, and light according to the degree of darkening on the echogram although the characteristics of a given layer were sometimes seen to change with time. A layer that appeared medium at 1 h may have become more dense during migration, appearing as a heavy layer later in the day. Such reclassification would occur only with heavy and medium layers. The light layers were quite distinct from the other two. Generally, however, it was possible to distinguish the three types of layers, and zooplankton catches confirmed that heavier layers usually had more animals per m^3 than light layers.

Zooplankton Sampling

Zooplankton samples were taken with a messenger-operated opening and closing ¼ m #6 (243-μ mesh) bongo net that took two independent samples simultaneously. The depth of the net was determined using a 12-kHz Benthos acoustic depth recorder with an accuracy of 25 cm at 100 m. The net was fitted with a flow meter that started to record the water flow when the net opened and stopped recording when the net was closed. Samples were preserved in a 5% solution of formalin and sea water. All euphausiids were removed from the samples, counted, identified to species, and their wet weights determined. The remaining zooplankton species were identified and wet weighed.

The ship was slowed to 3 knots when zooplankton samples were taken. The slower speed did not affect the intensity of the scattering layers on the echograms. Samples were taken in water showing no sound scattering layers at any depth, and also in water above and below the sound scattering layers. A total of 150 zooplankton samples were taken during the various cruises.

Chlorophyll Measurement

Chlorophyll *a* was measured using a continuous flow fluorometer by the methods described by Platt (1972) and Platt and Irwin (1972) during the surveys of June 1972 and May 1974. In 1973 1-liter water samples were filtered and the chlorophyll *a* on the filters measured by the methods of Yentsch and Menzel (1963). Chlorophyll *a* was measured at 5 m depth in 1972 and 2.5 m depth in 1973, and 1974. Chlorophyll *a* measurements were carried out along the same predetermined courses and simultaneously with the acoustic surveys.

Results

Sound scattering layers of different strengths as indicated by the darkness of the echograms were found along the north coastal area east of the Gaspé Peninsula during all surveys (Fig. 1A–E). An area of heavy scattering layers was found in June 1973, along the northern coast of the St. Lawrence River east of the Saguenay River and west of longitude 68° with medium scattering layers over most of the other parts of the estuary surveyed. There were no scattering layers in areas where the water was less than 100 m deep.

The coastal area of Gaspé from longitude 66° to 64° had scattering layers ranging from light to heavy with some areas having no scattering layers at all. The coast had heavy scattering layers during all the May and June cruises. The heaviest layering was found near the coast with the layers becoming lighter further from the coast towards the Laurentian Channel (Fig. 1A, B, and E).

A section of the northern coast of the Gulf was examined in June 1973 (Fig. 1B). Heavy scattering was found near the shore in water as shallow as 100 m. A zone of heavy to light scattering was located between Anticosti Island and the north shore of Quebec where the water was greater than 200 m.

During September 1973 the area between Anticosti Island and Gaspé had very light scattering layers with small patches of medium scattering

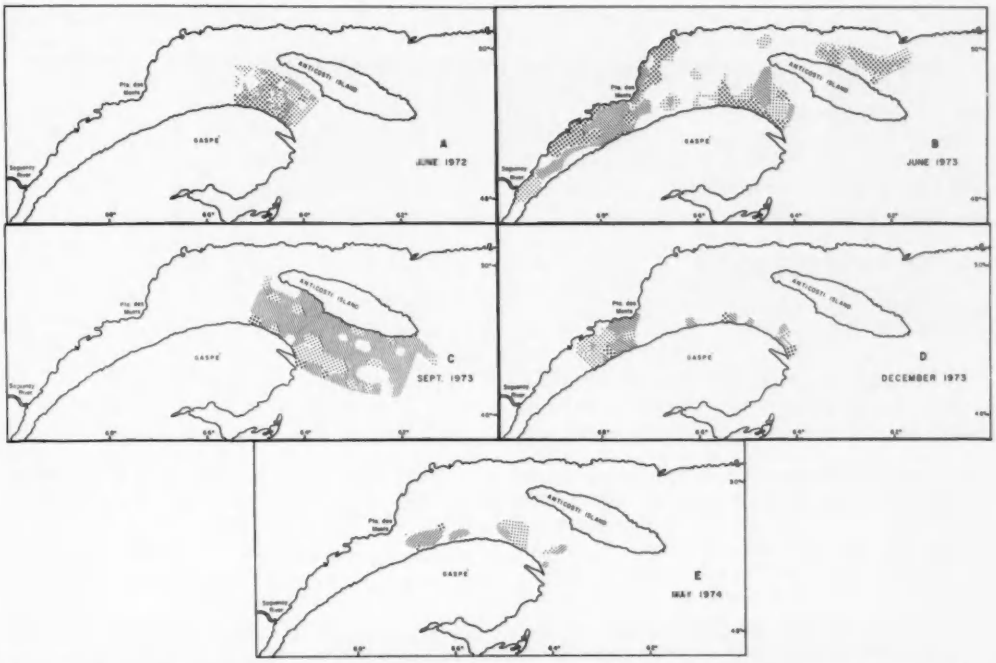

FIG. 1. Distribution of scattering layers during June 1972, June, September, and December 1973 and May 1974. Regions of heavy continuous scattering layers are large dots, medium scattering is represented by oblique lines, and light scattering by small dots.

and a few areas of heavy scattering near the Gaspé coast (Fig. 1C).

In December the Gaspé coast and the mouth of the St. Lawrence River were examined for scattering layers. Small areas of heavy to light scattering were found along the Gaspé coast and continuous scattering layers of heavy to light intensity were found in the river mouth (Fig. 1D).

BEHAVIOR OF SCATTERING LAYERS

The layers were usually found at a depth of 125–200 m from 1300 to 1400. At about 1500 they started to slowly ascend reaching a depth of 10–25 m by 0100 (Fig. 2). There was no evidence of sound scattering below 25 m from 0100 to 0300. The layers would start to sink at about 0400 and reached the maximum depth at 1300.

The thickness of the layers varied as they migrated to the surface. A layer that was 35 m thick at 100-m depth became concentrated into a layer about 5 m thick by the time it reached a depth of 25 m. A common feature in the scattering layers was the zonation of the main layer into a number of sublayers each about 1–5 m thick that appeared more dense than the areas between them. This was commonly seen in the area of heavy scattering along the Gaspé coast. The water in the region where heavy scattering was found in June 1972–73 usually contained three distinct temperature layers. A layer of about 0 C water was generally present at a depth of about 75–85 m. The water ranged from 2 C just above the 0 C zone to 9 C at the surface. In the top 5 m the temperature dropped from 9 to 4 C. The water below the cold layer ranged from 2 to 4 C. It appeared that where this temperature structure was distinct the animals causing the scattering layer were situated in the cold layer of water during the day. When there was no central cold layer the scattering layer was found at a greater depth.

ZOOPLANKTON COMPOSITION OF SCATTERING LAYERS

The zooplankton samples taken in the scattering layers always contained large numbers of euphausiids as well as copepods and, frequently the chaetognath *Sagitta elegans*, whereas samples

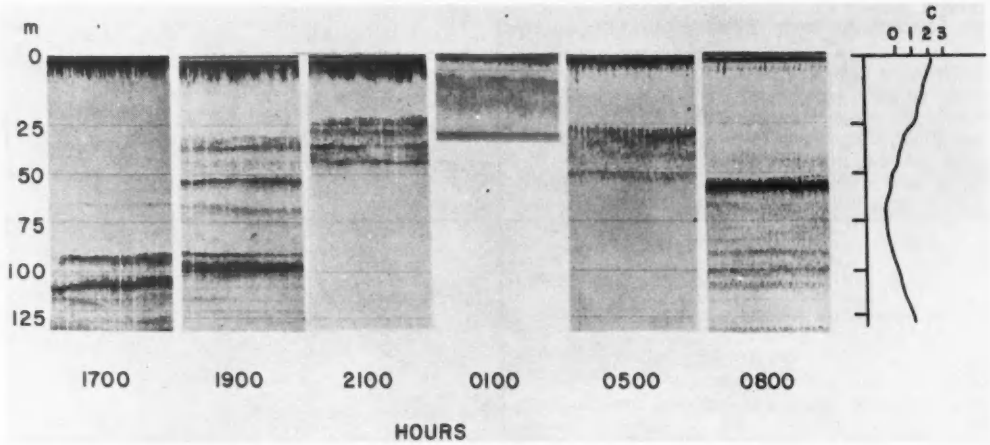

FIG. 2. Photos of echograms of migrating scattering layer at one station near the Gaspé coast. Temperature profile is shown on the right. Layer at 0100 was recorded with the upward looking sonar.

TABLE 1. Mean number ± SD, mean biomass ± SD of euphausiids, mean biomass ± SD of copepods, and *Sagitta* per m^3 in the different categories of scattering layers for the different sampling times.

	Category of scattering layer				Mean dry body wt of euphausiid (*g*)
	Nil	Light	Medium	Heavy	
		June 1972			
Euphausiids no.	0	.15± .17	1.4±0.9	4.5±1.4	
(*g*)	0	.02± .01	.19± .008	.41± .22	0.10
Copepods (*g*)	.20± .13	.80±1.08	.19± .13	.58± .79	
Sagitta (*g*)	.025± .037	.022± .025	.005± .002	.039± .058	
		June 1973			
Euphausiids no.	0	.48±44	2.0±1.1	9.1±4.3	
(*g*)	0	.07± .06	.18± .11	0.82± .50	0.10
Copepods (*g*)	.80±1.48	.34± .26	.28± .23	.45± .35	
Sagitta (*g*)	.001± .002	.0081± .01	.007± .01	.02± .02	
		Sept. 1973			
Euphausiids no.	0	.17± .28	.80± .53	36.5±21.8	
(*g*)	0	.01± .01	.09± .09	2.4±1.1	.066
Copepods (*g*)	.04± .02	.20± .13	.08± .10	.11± .06	
Sagitta (*g*)	.001± .0001	.028± .03	.009± .004	.008± .01	
		Dec. 1973			
Euphausiids no.	0	.89± .80	3.5±2.6	22.6±19.2	
(*g*)	0	.02± .02	.15± .12	.71± .10	.032
Copepods (*g*)	.004± .003	.01± .01	.007± .005	.01± .02	
Sagitta (*g*)	.0004± .0007	.001± .003	.002± .003	.001± .002	
		May 1974			
Euphausiids no.	0	.21± .07	3.1±1.6	27.1±20.2	
(*g*)	0	.05± .03	.34±11	1.21± .81	.044
Copepods (*g*)	.004± .005	.18± .21	.13± .11	.48± .38	
Sagitta (*g*)	0	.007± .007	.04± .06	.03± .05	

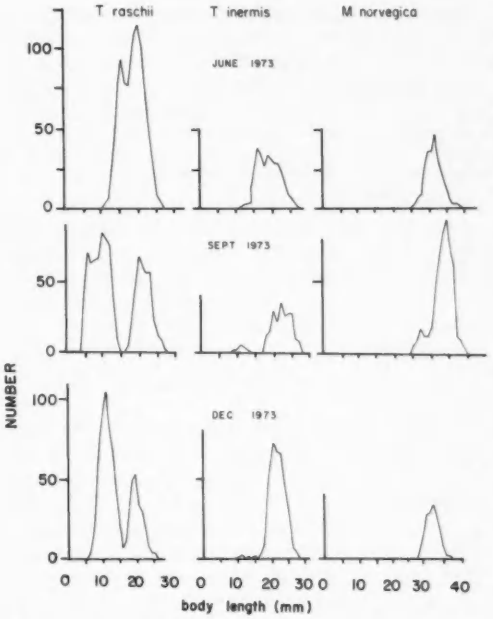

FIG. 3. Body length distribution of three species of euphausiids collected during the three cruises in 1973.

taken above and below the layers in what appeared on the echograms to be water void of scattering organisms had few or no euphausiids, but did not contain copepods and *Sagitta* in numbers as great or greater than in the scattering layer (Table 1). Samples taken in areas where there was no evidence of scattering layers at any depth contained varying amounts of copepods, but no euphausiids. There was no significant correlation between the biomass m^{-3} of copepods or *Sagitta* and the intensity of the scattering layers.

Three species of euphausiids were caught in the layers: *Thysaneössa raschii*, *T. inermis*, and *Meganyctiphanes norvegica*. The body sizes of the different species caught during the 1973 surveys are shown in Fig. 3. The copepods ranged up to 5 mm in length. *Sagitta* varied in length from 15 to 30 mm.

The mean numbers and biomass of euphausiids m^{-3} in the different categories of scattering layers were significantly different, with the layers giving the most intense echograms having the largest number of animals m^{-3}. There was no significant difference in the biomass of copepods or chaetognaths between areas of water with no scattering layers and water with heavy scattering layers (Table 1).

The maximum number and biomass of euphausiids m^{-3} were taken in a scattering layer at 100 m in September. The sample consisted entirely of *T. raschii* and had a density of 67 animals m^{-3} and a biomass of 4.5 g wet weight $^{-3}$. *Thysanöessa inermis* was the dominant species in the 1972 survey area along the Gaspé coast while in the central part of the Laurentian channel *M. norvegica* dominated.

In June 1973 the most extensive coverage of the upper estuary was made and showed that geographical differences in the distribution of the three species of euphausiids existed. Once more *M. norvegica* was concentrated in the region of the Laurentian Channel between the tip of Gaspé and Anticosti Island, and was the most abundant species in the scattering layers in this area. It was also very common and numerous on the north side of the St. Lawrence estuary between the Saguenay River and Pte. des Monts. *Thysanöessa raschii* was the dominant species in the area along the north shore and in the Anticosti Channel as well as along the Gaspé coast, whereas *T. inermis* was dominant only on the eastern tip of Anticosti Island.

During September 1973 the dominant species was *T. raschii*, and was concentrated in heavy scattering layers along the Gaspé coast. Small concentrations of *M. norvegica* were found in the Laurentian Channel at this time.

In December 1973 the scattering layers sampled along the coast of Gaspé and in the mouth of the St. Lawrence River west of Pte. des Monts contained predominantly *T. raschii*. A similar situation occurred in May 1974. The ratios of the different species in samples taken in the scattering layers were

	Ratio		
Date	*T. raschii*	*T. inermis*	*M. norvegica*
June 1972	1	7.5	1.5
June 1973	5.8	2.1	1
Sept. 1973	416	1	1.9
Dec. 1973	165	12	1
May 1974	10	1	1.1

CHLOROPHYLL *a* CONCENTRATION AND SCATTERING LAYERS

The concentrations of chlorophyll *a* and the presence of scattering layers were significantly correlated (Table 2). The fluorometer data in

TABLE 2. The frequency of stations with various echogram intensities related to the chlorophyll *a* concentration in the top 5 m of water and the correlation coefficient (*r*) between the two.

	June 1972			June 1973 (*mg chlorophyll a* m^{-3})			May 1974		
	<1–1	2–6	7–13	<1–.5	.6–1	>1	<1–2	3–4	>4
				Station frequency					
Echogram intensity									
Nil	19	1	0	20	14	7	11	1	0
Light	26	14	0	7	11	3	16	0	0
Medium	7	11	3	4	10	2	10	9	4
Heavy	8	20	3	6	9	8	1	0	1
	$r = .45$, df = 110 sign at $P < .05$			$r = .17$, df = 99 sign at $P < .10$			$r = .47$, df = 51 sign at $P < .05$		

June 1972 and May 1974 clearly showed this relationship. The data collected during June 1973, using water samples taken on stations at 6-mile intervals, did not provide as clear a pattern between the scattering layers and chlorophyll concentration, but the correlation coefficient was significant at the 10% level. The low correlation was probably due to the narrow range of chlorophyll *a* concentrations encountered.

The areas with low chlorophyll *a* concentrations were more likely to have no scattering layers or only light layers than areas with high chlorophyll *a* concentrations. On stations with the highest chlorophyll *a* readings the scattering layers were always of medium or heavy intensity (Table 2).

The general conclusion drawn from the data was that the euphasiids were likely to be found in areas of high chlorophyll concentration relative to the surrounding water, but it was not possible to establish an absolute lower limit of chlorophyll concentration below which no euphausiid scattering layers would be found.

Discussion

The results of the zooplankton catches provided evidence that the organisms causing the scattering layers were euphausiids. It is possible that some other species that were never caught in the nets formed the layers and the euphausiids were only associated with them. This is unlikely, however, because the net captured capelin up to 20 cm in length making complete avoidance by such an organism highly improbable.

Barham (1963) found the siphonophore *Nanomia bijuga* responsible for the DSL recorded at a frequency of 12 kHz near San Diego, Cal. There is no record of siphonophores in the Gulf of St. Lawrence estuary at the times of year of the surveys (Shih et al. 1971). The fine mesh of the net would have retained them, had they been there.

Chlorophyll *a* was concentrated in local areas along the Gaspé coast. That the highest concentrations of euphausiids were found in the areas of high chlorophyll *a* was likely due to the animals seeking out areas of high food concentration. *T. raschii* and *T. inermis* are primarily phytoplankton feeders while *M. norvegica* is an omnimore feeding on phytoplankton and small zooplankton. How the animals located the high phytoplankton concentrations is not known. Possibly some physical characteristic of the water mass in the region that caused the increase in chlorophyll *a* levels also caused the euphausiids to concentrate there.

The three species of euphausiids concentrated along the Gaspé coast during May and June, a period when the primary production in the area was the highest of the year (Steven 1974). The population density of ephausiids was greatly reduced in this region during September and December possibly as a response to the reduced primary production (Steven 1974).

Estimates of the density of euphausiids in the scattering layers were only as good as the catching efficiency of the nets. The degree of avoidance of the nets by the different species of euphausiids was unknown. The heaviest concentrations of animals were usually not sampled or else the net passed through areas of heavy and light animal density thereby giving an average catch. Therefore, the density and biomass of euphausiids in the layers were likely underestimated.

This study gives a convincing demonstration of the usefulness of a high frequency sounder in the location of euphausiid concentrations. Once experience and confidence were established in

recognizing the characteristics of the scattering layers caused predominantly by euphausiids, it was possible to cover large areas in search of concentrations of euphausiids without taking large numbers of zooplankton samples.

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Electrophysiological Studies of Morpholine-Imprinted Coho Salmon (*Oncorhynchus kisutch*) and Rainbow Trout (*Salmo gairdneri*)

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Two groups of coho salmon (*Oncorhynchus kisutch*) were exposed to a 5×10^{-5} mg/liter concentration of a synthetic chemical, morpholine, for 4 wk during the smolting period, while two corresponding groups were left unexposed (controls). All groups were marked by finclipping and stocked into Lake Michigan near the mouth of Oak Creek (South Milwaukee, Wisconsin). A second series of exposed and unexposed salmon was released 13 km north of Oak Creek. Rainbow trout (*Salmo gairdneri*) were tested in a similar experiment.

Homing salmonids were censused and then used in physiological experiments. Electroencephalographic studies showed significantly greater responses by the experimental (exposed) compared to control fish to 1% morpholine ($P \leq 0.001$–0.05) and by one paired group to a stream sample scented with morpholine (about 10^{-3} mg/liter morpholine $P \leq 0.01$). Responses to morpholine were specific in that another chemical similar to it did not elicit responses. These data support the olfactory hypothesis that salmon may imprint to naturally occurring homestream odors in the same way as they do to morpholine.

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Nous avons exposé deux groupes de saumon coho (*Oncorhynchus kisutch*) à une concentration de 5×10^{-5} mg/litre d'un composé chimique synthétique, la morpholine, sur une période de 4 semaines durant la période de smoltification, alors que deux groupes correspondants n'ont pas été exposés (témoins). Nous avons marqué tous les groupes par ablation de nageoires et les avons relâchés dans le lac Michigan près de l'embouchure du ruisseau Oak (Milwaukee-Sud, Wisconsin). Nous avons relâché une seconde série de saumons exposés et non exposés à 13 km au nord du ruisseau Oak. Nous avons fait une expérience semblable avec des truites arc-en-ciel (*Salmo gairdneri*).

Nous avons compté les salmonidés retournant au cours d'eau et les avons ensuite utilisés dans des expériences physiologiques. Des études électroencéphalographiques démontrent que les poissons expérimentaux (exposés) réagissent beaucoup plus fortement à la morpholine à 1% que les poissons témoins ($P \leq 0.001$–0.05), et pour un groupe apparié, à un échantillon du cours d'eau imprégné de morpholine (environ 10^{-3} mg/litre de morpholine, $P \leq 0.01$). Les réponses à la morpholine sont spécifiques : une autre substance chimique semblable ne déclenche pas de réponses. Ces données appuient l'hypothèse olfactive selon laquelle les saumons conservent la mémoire d'odeurs naturelles du cours d'eau natal de la même façon que celle de la morpholine.

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SALMONID homing has been divided into two phases: 1) orientation in open water and 2) orientation in and near the homestream. It has been proposed that salmon are able to imprint to the odor of their homestream during the early stages of their life and recall this information during the stream phase of the homing migration (Hasler and Wisby 1951). Since the chemicals of importance to homing in the stream have not been identified (largely because of the low concentration and variety of these odors), Hasler and Wisby (1951) proposed that salmon fingerlings be imprinted to a synthetic odor and decoyed into a stream scented with the odor during the spawning season.

Several earlier field (census and ultrasonic tracking) and laboratory (EEG) reports have documented that coho salmon can be imprinted to morpholine (Cooper 1974; Cooper and Hasler 1973, 1974, 1975; Scholz et al. 1973; Madison et al. 1973). These studies showed that imprinted

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salmon homed specifically to a stream scented with morpholine and exhibited characteristic high amplitude brain signals when this chemical was put in the nares. Concurrent papers (Cooper et al. 1976; Cooper and Scholz 1976) confirm the results of the census experiments.

The objective of this study was to determine with a physiological correlate whether coho salmon (*Oncorhynchus kisutch*) and rainbow trout (*Salmo gairdneri*) can be imprinted to morpholine (an odorous substance) and retain this information until the spawning migration. Further questions about the validity of the EEG technique as a laboratory bioassay will also be explored.

Materials and Methods

Four groups of 5000 coho salmon fingerlings each were held at Wild Rose Fish Hatchery beginning in April 1972. All groups were marked by finclipping. Two groups were exposed to morpholine at a concentration of 5×10^{-5} mg/liter, while two groups were left unexposed. The two experimental groups were exposed for 5 wk, 3 wk before until 2 wk after smolting behavior was observed. One experimental and an unexposed group were transported by truck and released near Oak Creek, South Milwaukee. The other pair of experimental and control fish was released 13 km to the north of Oak Creek at Milwaukee Harbor (fig. 1, Cooper et al. 1976).

Two additional groups of 5000 fish were held at South Milwaukee in tanks supplied with Lake Michigan water. One group was exposed to morpholine at 5×10^{-5} mg/liter, while the other was left unexposed. These two groups were then released near Oak Creek.

An additional experiment with rainbow trout was also conducted (Cooper and Scholz 1976). Three groups of rainbow trout were held at Oak Creek for about 1 mo. One group was exposed to morpholine at a concentration of 5×10^{-5} mg/liter, while the other two were left unexposed to serve as controls. The exposed group and one control group were released 13 km north of Oak Creek at the Milwaukee Harbor, while the second control group was released at Oak Creek. The experimental design for two groups released near the Milwaukee Harbor was similar to that of the coho salmon experiment 2b. A summary of these experiments is given below (Cooper et al. 1976; Cooper and Scholz 1976).

Experiment	Group	Imprinting location	Release site
2a	morpholine	South Milwaukee	Oak Creek (S. Milwaukee)
	control		
2b	morpholine	Wild Rose Fish Hatchery	Milwaukee
	control		
2c	morpholine	Wild Rose Fish Hatchery	Oak Creek (S. Milwaukee)
	control		
trout	morpholine	South Milwaukee	Milwaukee
	control		Milwaukee
	control		Oak Creek

In the fall 1973 spawning season when the adults from the 1972 stocking became mature, morpholine was dripped into Oak Creek to produce a concentration of about 5×10^{-5} mg/liter (the concentration used previously for imprinting). We hypothesized that if the fish were homing to the odor, the exposed fish would return to the scented stream in greater numbers than would the controls. Additionally, if the areas searched by the salmon during the second phase of the homing migration were large enough, then the experimental group released 13 km to the north might also locate and home to Oak Creek.

Large numbers of salmon and trout were captured at Oak Creek in the fall 1973 by several methods including electrofishing and gillnetting. Four fish were tested each day using the electroencephalographic (EEG) technique. In this technique, previously described (Cooper 1974; Cooper and Hasler 1973, 1974), an electrode was inserted in the olfactory bulb and brain signals recorded with a polygraph. Concentric electrodes (SNEX-100, David Kopf) were used instead of coaxial electrodes in these studies. Both kinds of control fish were used in the rainbow trout experiment — those released at the Milwaukee Breakwater and those released near Oak Creek. Both are equally valid in comparing responses to morpholine from the imprinted groups, since neither of the control groups had any previous exposure during smolting to morpholine.

Immobilized fish were tested with a total of 22 test solutions in a random sequence, each sample followed by a dechlorinated tap water rinse. These sequences were replicated 3 times for most fish. Some of these water samples were similar to those used in a previous study (Cooper and Hasler 1974).

Different concentrations of morpholine (1, 0.1, and 0.01%) were used to test the responses of the fish to the imprinting chemicals. Solutions of morpholine (1, 0.1, and 0.01%) buffered with sodium bicarbonate to pH 7.5 were tested to control for pH, which is a factor in the EEG response (Cooper and Hasler 1973; Dizon 1971; Dizon et al. 1973). Phenethyl alcohol at concentrations of 1, 0.1, and 0.01% was used to determine whether an organic compound other than morpholine elicited responses. Three buffered solutions at these concentrations were also used. Lake Michigan and Oak Creek water (above the point were the morpholine was introduced) were tested to determine the influence of water which the fish had most recently experienced prior to capture and to test for the presence of nonspecific stimulatory products. Buffer (0.01 M) was used to control for ionic strength.

Three solutions of N-β-hydroxethylmorpholine (at concentrations of 1, 0.1, and 0.01%), a compound closely related to morpholine, were tested to determine the specificity of the response to morpholine. A sample of Oak Creek water taken below the place where morpholine was introduced was also tested. In this area the morpholine concentration was roughly 10^{-3} mg/liter. Sodium chloride (0.35%) was used as standard solution in these experiments. L-methionine

TABLE 1. Significant differences in responses of three groups of morpholine-exposed (experimental) and unexposed (control) coho salmon (*Oncorhynchus kisutch*) and a paired group of exposed and unexposed rainbow trout (*Salmo gairdneri*) to 1% (10^4 mg/liter) morpholine, Oak Creek–morpholine (stream water scented with morpholine at about 10^{-3} mg/liter) and L-methionine, 10^{-5} M. All are compared to the response to NaCl (0.35%). Significance is determined by the Mann–Whitney *U* test. NS = not significant.

Solution	Salmon			Trout
	Exp. 2a	Exp. 2b	Exp. 2c	
1% morpholine	≤ .05	< .001	≤ .001	≤ .025
Oak Creek–morpholine	NS	≤ .01	NS	—[a]
L-methionine (10^{-5} M)	NS	NS	≤ .05	NS

[a]Not tested.

(10^{-5} M) was used to test for basic differences in responsiveness of the two groups.

The EEG records were standardized by dividing the integration of the response to each test solution by the integration of the NaCl and, separately, the L-methionine records. Differences in the means of the responses for all fish were evaluated for significance by the Mann–Whitney *U* test (Siegel 1956); responses that were separated by less than 0.03 were considered the same rank.

Results

Exposure to morpholine during the smolting period significantly influenced the subsequent neural response to this substance. Thus, exposed salmonids were considered to be imprinted to morpholine. There was a significant difference in response between imprinted and control groups to 1% (10^4 mg/liter) morpholine for all three salmon experiments (Table 1) and for the rainbow trout experiment. (Information is summarized in Table 1 and can be found in detail in Table 2 and Cooper 1974.) There was a significant difference in response to the Oak Creek–morpholine solution for the exposed and unexposed fish in experiment 2c, but not for fish in the other two experiments. Trout were not tested with Oak Creek–morpholine solutions.

To test whether the difference in response to morpholine was a result of a basic difference in responsiveness of the imprinted and nonimprinted groups, the response to another olfactory substance, L-methionine, for these two groups was also compared. Responses to this substance were not significantly different for the fish in experiments 2a, 2b or in the rainbow trout experiment; fish in the third showed a difference at the 0.05 level (NaCl standard). Thus, differences in responsiveness to morpholine may be due partly to a basic difference in responsiveness of the latter.

No significant responses to other substances were observed, including the weaker morpholine solutions, buffer, Oak Creek water near the dam, or buffered morpholine solutions. Since there were no responses to either N-β-hydroxyethylmorpholine or phenethyl alcohol, we conclude that the responses to morpholine were specific. As there were few responses to the Oak Creek water sample, we feel that the situation supports the earlier findings that recent exposure, such as residence time in the river prior to capture, is not an important factor during the spawning season (Cooper and Hasler 1974).

Examples of EEG responses to some of these water samples are given in Fig. 1. It must be emphasized that there are no typical responses. Large numbers of records must be examined to produce statistically valid numbers.

Discussion

Whatever the nature of the response (i.e. whether by olfaction or another sensory system), highly significant and consistent differences are seen in responses to morpholine for fish previously exposed vs. not exposed to morpholine as juveniles. Thus, the EEG experiments provide evidence leading to the conclusion that the previous exposure to morpholine (at an extremely low concentration) significantly influences the subsequent electrical responses recorded from the olfactory bulb. This may be a mechanism of the central nervous system of memory recall or only a correlate that happens to simulate quite closely the observed behavior of the animal. This experiment has been performed on four paired groups of 90 coho salmon as well as one paired group of rainbow trout over a period of 2 yr, so that results are consistent (Cooper and Hasler 1974). Dizon (1971) and Dizon et al. (1973) obtained similar results. This is the main point of our experiments.

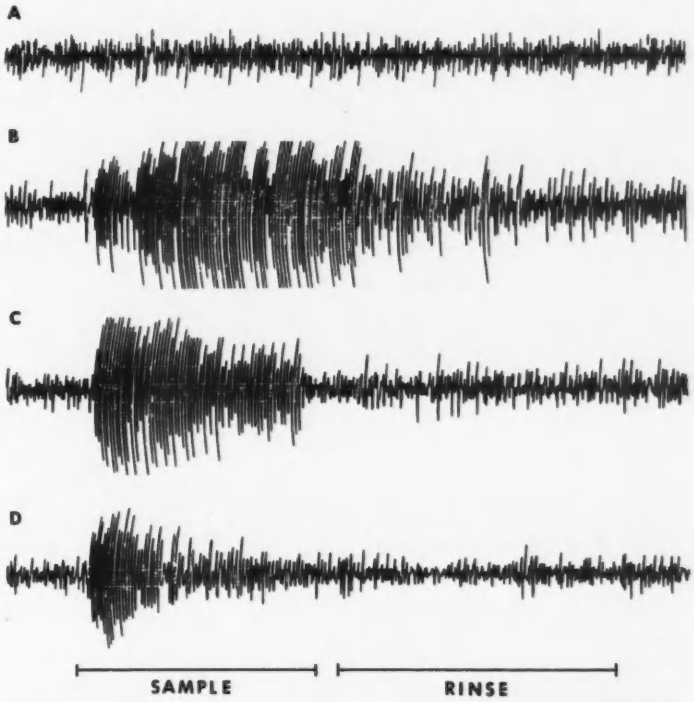

FIG. 1. Examples of EEG responses. A, Nonimprinted fish response to 1% morpholine; B, Imprinted fish response to 1% morpholine; C, Imprinted fish response to L-methionine; D, Imprinted fish response to Oak Creek water scented with morpholine (at a concentration of about 1×10^{-8} mg/liter).

Since there have been questions about the nature of the EEG responses to morpholine (Hara 1974; Hara and Macdonald 1975), this issue will be explored here. In these articles it was suggested that responses are due to some nonolfactory system, perhaps due to high pH or a general irritant effect of 1% morpholine solutions. Evidence for this included 1) an inhibitory effect on responses to L-serine after repeated applications of 1% morpholine to the nares, 2) inhibitions in the background (spontaneous) EEG levels, 3) qualitative differences in responses to morpholine compared to responses to other substances, and 4) the high concentrations of morpholine needed to elicit responses.

We do assume that morpholine is detected by olfaction. If responses were due to pH or some other general irritant effect (Hara 1974; Hara and Macdonald 1975), then one might expect no difference in responses between imprinted and control fish. As reported in this paper there were highly significant differences in responses to morpholine for these two groups.

These recent experiments again confirm that responses to morpholine are not owing to a nonspecific response such as pH. Cooper and Hasler (1974) demonstrated strong responses to buffer and morpholine, although there were no responses to a pH buffered morpholine solution. We concluded that buffer and morpholine had counteracting effects. No responses to buffer were seen in the present work because it was used at a concentration of 0.01 M in contrast with 0.1 M in the earlier work. In addition, earlier experiments (Cooper and Hasler 1973, 1974) show that there were no significant differences in responses to a sodium bicarbonate buffer solution at pH 9.5 for imprinted and nonimprinted fish.

Unlike other reports (Hara and Macdonald 1975; Hara 1974), there also were no decreases in responses to an amino acid (in this case, L-methionine instead of L-serine) after the introduction of 1% morpholine. Responses to L-methionine were fairly consistent for a given fish and after three trials with 1% morpholine were not significantly different than responses

TABLE 2. Responses to 1% morpholine, Oak Creek–morpholine (stream water scented with morpholine), and L-methionine by morpholine-imprinted (M) and nonimprinted (C) coho. All are compared to the response to NaCl 0.06 M (0.35%). All three or more trials, except * = two trials, ( ) = one trial. Rank and significance are determined by the Mann–Whitney *U* test. NS = not significant.

		1% Morpholine		Oak Creek–Morpholine		L-methionine 10^{-5} M	
Group	Date	Rank	Response	Rank	Response	Rank	Response
			Experiment 2a				
C	Nov. 5	1	1.00	6	1.00	8.5	1.45
C	Nov. 13	2	1.04	6	1.00	1	.90
C	Oct. 26	3	1.34	6	1.00	3.5	1.26
C	Nov. 15	4.5	1.45	6	1.00	13	2.10
M*	Nov. 15	4.5	1.46	6	1.00	8.5	1.43
C	Nov. 8	6	1.69	19	2.15	10	1.58
C	Oct. 31	7	1.96	6	1.00	16	2.63
M	Nov. 7	8	2.00	12	1.08	2	1.19
M*	Nov. 8	9.5	2.11	16	1.61	7	1.36
C*	Nov. 7	9.5	2.14	6	1.00	3.5	1.25
M	Nov. 16	11	2.32	17.5	1.68	5.5	1.31
M	Oct. 25	12	2.48	6	1.00	11	1.93
C	Nov. 1	13	2.63	20	2.36	16	2.65
M	Oct. 30	14	2.67	13	1.21	16	2.66
M	Oct. 26	15	2.85	15	1.38	21	3.98
C	Oct. 30	16.5	3.11	6	1.00	20	3.86
M	Nov. 13	16.5	3.14	14	1.29	19	3.33
(M)	Oct. 28	18	3.34	6	1.00	23	4.24
M	Nov. 19	19.5	3.50	6	1.00	12	2.00
C	Nov. 16	19.5	3.50	23	3.71	22	4.08
M	Oct. 24	21	3.66	17.5	1.65	5.5	1.30
C	Nov. 19	22	4.02	21	3.01	18	3.03
M	Oct. 14	23	4.51	22	3.06	14	2.21
		$U = 38$		$U = 58$		$U = 80$	
		$P \leq .05$		$P > .05$ (NS)		$P > .05$ (NS)	

seen before trials began ($N = 3$, $P > 0.05$, Siegel 1956). Thus, no trend towards decreasing responsiveness was seen.

There were no responses to 0.1 and 0.01% morpholine; neither was there an inhibition in the background by this chemical as described by Hara (1974) and Hara and Macdonald (1975). During three trials some decreases in background activity were seen, but were well within the expected variation for the number of trout tested (14 fish, 40 trials). Thus, the results of Hara and Hara and Macdonald may be due to a small sample size, although they do not give this information.

Certainly the responses to morpholine are qualitatively different from responses to other substances (Cooper and Hasler 1974). It should not be assumed, however, that there are no differences qualitatively to "other" substances, since some have been observed (Cooper 1974). While Hara (1974) and Hara and Macdonald (1975) assume this qualitative difference in the response to morpholine is due to some nonolfactory response, we feel that this kind of evoked potential indicates the response to a substance with great biological significance for the salmon and trout.

Hara (1974) and Hara and Macdonald (1975) had to repeat the application of morpholine to elicit strong responses from nonmigratory, sexually imature rainbow trout. Such a priming was not necessary with the imprinted rainbow trout or coho salmon. For nonimprinted trout that did respond, greater responses were sometimes seen at the end of an experiment, although morpholine had been tested several times previously.

We acknowledge that 1% morpholine, the concentration used in the EEG technique, is extremely high when compared with the imprinting concentration of 5×10^{-5} mg/liter. This problem of threshold is not unique. As reported earlier (Cooper and Hasler 1974), Hara (1972) found that the EEG threshold for L-serine is 10^8 times higher than that observed in behavioral

TABLE 2. (*Concluded*)

Group	Date	1% Morpholine		Oak Creek–Morpholine		L-methionine 10^{-5} M	
		Rank	Response	Rank	Response	Rank	Response
				Experiment 2b			
C	Nov. 6	1	.95	4.5	1.00	7	1.45
C	Nov. 17	2.5	1.00	4.5	1.00	4	1.31
C	Nov. 14	2.5	1.00	4.5	1.00	8	1.75
C	Nov. 13	4	1.04	4.5	1.00	1	.90
C*	Nov. 8	5.5	1.21	12.5	1.21	4	1.30
C	Oct. 28	5.5	1.22	4.5	1.00	4	1.27
C	Nov. 4	7	1.79	4.5	1.00	21	3.18
M	Oct. 9	8	1.97	11	1.12	4	1.24
M	Nov. 17	9	2.03	9.5	1.07	13	2.07
C	Oct. 9	10	2.54	14.5	1.38	15	2.35
M	Oct. 28	11	2.73	12.5	1.22	16	2.64
M	Nov. 6	12	2.94	19	2.24	14	2.25
M*	Oct. 24	13	3.05	18	2.10	11.5	1.97
M	Nov. 3	14	3.13	20.5	2.71	20	3.12
M	Nov. 8	15	3.35	20.5	2.70	4	1.28
(M)	Nov. 4	16.5	3.41	22	3.41	18.5	3.00
M	Nov. 14	16.5	3.41	4.5	1.00	18.5	3.00
M*	Nov. 1	18	3.63	4.5	1.00	9	1.80
C	Oct. 28	19	3.68	9.5	1.07	17	2.93
M	Oct. 3	20	3.93	17	1.80	10	1.90
M	Oct. 4	21.5	3.98	14.5	1.37	22	3.25
M	Oct. 4	21.5	4.01	16	1.43	11.5	1.97
		$U = 12$		$U = 18.5$		$U = 36$	
		$P \leq .001$		$P \leq .01$		$P > .05$ (NS)	
				Experiment 2c			
C	Nov. 19	1	1.06	13	1.25	5	1.35
C	Nov. 12	2	1.21	5.5	1.03	8.5	1.59
C	Oct. 18	3	1.26	18	1.75	13	2.26
C*	Nov. 5	4.5	2.02	5.5	1.00	5	1.32
C	Oct. 25	4.5	2.02	12	1.20	2	1.20
C	Nov. 7	6.5	2.06	21.5	1.91	1	1.00
M*	Nov. 16	6.5	2.06	16.5	1.46	5	1.38
M	Oct. 24	8	2.10	19.5	1.87	11	1.81
C*	Nov. 4	9.5	2.20	25	5.50	18.5	2.76
C	Nov. 15	9.5	2.22	5.5	1.00	10	1.75
M	Nov. 19	11	2.26	19.5	1.88	3	1.26
M	Oct. 31	12	2.44	5.5	1.00	14.5	2.30
C	Oct. 17	13	2.53	15	1.33	12	2.16
M	Oct. 24	14	2.67	23.5	2.13	7	1.46
C	Oct. 25	16	2.78	5.5	1.00	17	2.64
C	Nov. 14	16	2.80	5.5	1.00	18.5	2.79
M	Oct. 17	16	2.83	11	1.12	20	3.24
M*	Nov. 5	18	2.90	5.5	1.00	8.5	1.62
M	Nov. 15	19	2.99	5.5	1.00	14.5	2.32
M	Nov. 12	20	3.14	14	1.29	21.5	3.33
M*	Oct. 16	21	3.36	23.5	2.16	23.5	3.92
M	Nov. 14	22	3.70	5.5	1.00	16	2.47
M	Oct. 13	23	4.12	16.5	1.47	25	3.98
M*	Oct. 31	24	4.72	5.5	1.00	23.5	3.94
M	Oct. 25	25	4.81	21.5	1.90	21.5	3.32
		$U = 19.5$		$U = 65.0$		$U = 44.5$	
		$P \leq .001$		$P > .05$ (NS)		$P \leq .05$	

experiments. We detected significant differences in evoked potentials in response to stream water scented with morpholine at about 10^{-3} mg/liter, a concentration 10^3 times higher than that used in behavioral experiments (Cooper 1974; Cooper and Hasler 1975b). Therefore, responses to morpholine show the same difference in threshold as do responses to amino acids and other chemicals tested by Hara. The EEG technique does not appear to be reliable when working in the low concentration range because thresholds determined by physiological means are often higher than those observed in behavioral tests (Cooper and Hasler 1974).

Although ablation studies are needed to resolve whether morpholine is detected by olfaction, there is ample behavioral evidence that olfaction is important in this system. Morpholine is detected at very low concentrations both in a laboratory study (Wisby 1952) and field research (Madison et al. 1973; Scholz et al. 1973). A concentration of 5×10^{-5} mg/liter is sufficient for imprinting juvenile coho salmon and rainbow trout and for decoying these fish to a stream scented with morpholine. Since no other sensory system is known to operate at such low concentrations, olfaction is considered to be the sensory modality. Recent research (Tucker 1973; B. Oakley personal communication) indicates that gustatory systems of some fish are extremely sensitive so that this system might also be considered, although high sensitivity of the gustatory system has not been demonstrated for salmonids.

Results offered with the EEG technique are in close agreement with other parts of our studies (Cooper et al. 1976). We conclude that coho salmon and rainbow trout can imprint to morpholine, retain this information until the spawning migration, and use it to identify a stream scented with this chemical. The response to this odor is specific in that a compound similar to morpholine (N-β-hydroxyethylmorpholine) and another organic chemical odor (phenyl alcohol) failed to elicit responses. These data provide support for the olfactory hypothesis: salmon may imprint to morpholine in the same way as they do naturally occurring homestream odors.

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Agonistic Behavior of Captive Goldeye (*Hiodon alosoides*)

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FERNET, D. A., AND R. J. F. SMITH. 1976. Agonistic behavior of captive goldeye (*Hiodon alosoides*). J. Fish. Res. Board Can. 33: 695–702.

Captive goldeye (*Hiodon alosoides*) (ages 0, I, V–VIII) in clearwater tanks demonstrated a well-developed agonistic repertoire which included charging, chasing, nipping, and lateral displays. We postulate that this agonistic behavior serves as a spacing mechanism in goldeye ecology, allowing fish to defend an individual distance or territory. Age 0 and age I fish showed higher frequencies of direct attack than adults (> age V) which used displays more frequently. In paired encounters, females, smaller fish, and introduced fish won more fights than males, larger fish, or resident fish, a situation which differs from the usually accepted relationships between fighting success and size, sex, or residency. Ripe adults were less aggressive than nonripe adults.

FERNET, D. A., AND R. J. F. SMITH. 1976. Agonistic behavior of captive goldeye (*Hiodon alosoides*). J. Fish. Res. Board Can. 33: 695–702.

Des laquaiches aux yeux d'or (*Hiodon alosoides*) (d'âges 0, I, V–VIII) maintenues en captivité dans des réservoirs d'eau claire font preuve d'un répertoire bien développé d'activités combatives, dont l'attaque, la poursuite, les petites morsures et la parade. Cette combativité servirait de mécanisme d'espacement dans l'écologie de la laquaiche aux yeux d'or, permettant à un poisson de maintenir les autres à distance et de défendre un territoire propre. L'attaque directe est plus fréquente chez les poissons d'âges 0 et I que chez les adultes (> âge V). Chez ces derniers, la parade est l'activité la plus fréquente. Dans les rencontres entre sexes, les femelles, les petits poissons et les poissons introduits sont plus souvent vainqueurs que les mâles, les grands poissons et les poissons résidents. Cette situation diffère des relations généralement reconnues entre le succès au combat d'une part et la taille, le sexe ou le temps de résidence d'autre part. Les adultes mûrs sont moins agressifs que les adultes non mûrs.

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LITTLE is known about the behavior of goldeye (*Hiodon alosoides*); observation in their turbid habitat is difficult or impossible, and they are too large to be convenient laboratory subjects (Kennedy and Sprules 1967; Fernet 1973). Descriptions of hiodontid agonistic and reproductive behavior are nonexistent in the literature. We have documented the behavior of age 0, I, and adult (>V) goldeye observed in clearwater aquaria and window-equipped tanks, and attempted to relate the behavior of this economically important species to the reported aspects of goldeye ecology and life history. Spawning behavior was not observed in our tanks, but differences in the agonistic behavior of ripe and nonripe adults are reported.

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Materials and Methods

We captured over 600 goldeye in the field, transported them to holding and observation facilities, and observed their behavior for over 200 h under a variety of environmental and social conditions. Capture and most observation of fish took place during the spring, summer, and fall months. Most of the age 0 fish were captured and observed outdoors in the Peace–Athabasca Delta, Alberta. Age I and adult fish were captured primarily in the South Saskatchewan River and observed in both indoor and outdoor tanks in Saskatoon.

During transport the fish were treated with NaCl and tetracycline. We held the goldeye in 360-liter stream tanks or 18-kl pools, with aeration and flowing water, prior to observing them. Temperatures during holding and observation were maintained close to the South Saskatchewan River temperatures at Saskatoon (3–22 C, seasonal range). Age 0 fish were observed in 45-liter aquaria, age I fish in 135-liter aquaria, and adults in both 3.5- and 18-kl tanks. A variety of live and dead food was presented to goldeye, but they seldom ate in captivity. They had large fat reserves when captured and were apparently affected little by starvation. Although all observations

TABLE 1. Conditions for quantitative 30-min observations, in order of discussion in text.

Age	Temp (C)	Photoperiod	Day illumination (lx)	Tank vol (liters)	Group size observed	Sex combinations	Size: Fork length (cm)	Size: Wt (g)	Total no. observed	Total h observation	Time of observation
A VI+	15–19.5	16.8L:7.2D	8.6–172.2[a]	3,500	2	♂–♂ ♀–♀ ♀–♂ ♂–♀♀ ♂–♀	34.1–34.6 35.9–34.9 42.0–31.5 35.0–39.5 34.5–38.5	518–537 558–533 1023–363 503–875 494–689	10	20	0930, 1400, 1730, 2230[b]
		15L:9D									
B VI+	Ripe 15–18	17L:7D	8.6–172.2[a]	3,500	2				6	9	0930, 1400, 2100
			4.3[c,d]	3,500	2	♂–♀	—	—	8	15.5	0930, 1400, 1930[b]
		15.5L:8.5D	8.6–172.2[a]	18,500	2				8	17.5	1000, 1430, 2130[b]
C O	15–24	18L:6D	688–2691[a]	45	2	Immatures Immatures Immatures	5.0–5.1 5.9–5.8 7.3–7.6	1.3–1.4 2.4–2.3 4.5–4.6	6	12.5	0900, 1600
		16.25L:7.75D									
D I	14–19	17L:7D	53.8[c,d]	135	2	Immatures Immatures Immatures	15.6–15.8 15.7–16.0 15.7–16.3	39.7–40.2 40.0–43.1 39.9–45.3	6	8.5	0930, 1600
		16.5L:7.5D									
E VI+	15–19	17L:7D 16.5L:7.5D 18L:6D	8.6–172.2[a]	3,500	2	♂–♀	—	—	8	13.5	0930, 1400, 2200[b]
F O	15–24	16.25L:7.75D	688–2961[d]	45	3	Immatures Immatures	5.1–5.0–5.0 7.6–7.6–7.5	1.4–1.3–1.4 4.7–4.6–4.6	6	11.5	0900, 1600
G VI+	15–19.5	16.8L:7.2D	8.6–172.2[a]	3,500	3	♀–♀–♀ ♀–♀–♂ ♀–♂–♂ ♂–♀–♂ ♂–♀–♀	39.0–33.5–37.3 37.0–39.2–35.1 39.5–35.8–34.4 36.6–38.2–35.3 35.5–35.0–34.8	705–520–662 674–1082–637 743–538–525 642–715–488 561–457–471	15	10	0930, 1400, 1730, 2230[b]
		15L:9D									

[a]Measured through viewing window.
[b]Used red fluorescent light, < 1.1 lx at viewing window.
[c]Indoor, artificial illumination.
[d]Measured at water surface.

reported here were made within a few weeks of capture, some individuals survived without eating for over 1 yr and still appeared to be in good health.

Observation Conditions

All observational periods reported here (Table 1) were 30 min long, recorded on magnetic audio tape and later transcribed. Small blinds concealed the observer during the majority of observations. For indoor tanks, photoperiods were adjusted to match the natural cycle. Tanks were illuminated with red fluorescent lights (<1.1 lx) during night observations. Qualitative and quantitative observations were carried out under a variety of conditions in addition to those summarized in Table 1. These preliminary observations were used to formulate an ethogram, a prerequisite for quantification of behavior, to establish suitable methods for quantification, and to check for behavioral differences related to variations in environmental conditions.

Following development of an ethogram and of suitable criteria for dominance based on the ethogram, we began quantitative analysis of the behavior of the three age-groups of goldeye which were readily available to us. Age 0 goldeye were observed in pairs and trios for 12.5 consecutive days. Lack of facilities in the Peace–Athabasca Delta prevented prior isolation. Age I fish were isolated for 48 h before observation. Adult fish were isolated in stream tanks 48 h before observation, with one fish (the resident) being moved to the observation tank 10 h before the first observation. Others (introduced fish) were placed in the tank at 0930 just before the observation began. Pairs were observed on 2 consecutive days; trios for 1 day only. Fish were weighed and measured after each set of observations was completed. All combinations of residency and sex were observed for adult pairs and trios.

Five pairs of adults (Table 1A) were observed for a total of 20 h with each pair being observed for 4 h over a 2-day period. We compared the frequencies of seven types of behavior patterns: charge, nip, chase, approach, lateral display, avoidance, and fleeing. Approach–charge was combined with charge, and attempted nip with nip in this and all subsequent analysis. Fish were categorized according to size, sex, and residency. Differences between categories in the frequency of each behavior pattern were analyzed using a χ^2 analysis (Sokal and Rohlf 1969).

The pattern of differences among categories was visualized by assigning ranks (1–7) to the differences between the frequencies of each of the seven behavior patterns within each comparison of categories (male–female, large–small, resident–nonresident). These ranks were rated positive if the difference favored the dominant fish and negative if the difference favored the subordinate. This nonparametric technique of ranking differences, then rating the rank + or − on the basis of the direction of the difference, is suitable for data measured at the level of an ordered metric scale (Siegel 1956). Such data cannot be legitimately analyzed with parametric statistics, a common situation with behavioral data.

To document the spatial distribution of adults, we plotted the position of each member of seven pairs of ripe fish (Table 1B) and four pairs of sexually regressed adult goldeye (Table 1E) in 3½- and 18-kl tanks at 2½-min intervals during 70 observations. The location of each interaction was also plotted along with information on which fish initiated the interaction.

Ripe adults were diagnosed by squeezing milt from males and observing the ovaries of females through an otoscope inserted in the urogenital opening (Driscoll 1969). Ripe heterosexual pairs were observed without prior isolation.

Results

Agonistic Ethogram

Goldeye swim continuously, maintaining a fairly constant speed which we have termed "normal speed." During interactions swimming speed is varied in a characteristic manner for each action, for example by speeding up for a charge or matching the opponent's speed for a lateral display. Goldeye behavior resembles the free-flowing behavior of salmonids and cyprinids rather than the intermittent behavior of stickleback or centrarchids.

Approach — One fish moves straight toward another at normal speed, usually approaching from the side or rear.

Following — One fish swims behind another, maintaining a constant distance between fish. Occasionally, following was performed simultaneously by two fish that swam in a circle, head to tail.

Charge — One fish moves swiftly and directly towards its opponent, sometimes with its mouth open and fins spread.

Approach–charge — Initially a fish approaches its opponent at normal speed, then as it nears its opponent increases to a speed typical of charging. The mouth may be opened and fins spread, as in charging.

Lateral display — A fish maintains a position, usually above its opponent, in which its flat side is nearly perpendicular to the opponent's line of sight. (Goldeye are laterally compressed.) Variations occur: i) a fish may glide beside another with only the paired fins spread or with all fins spread, ii) a fish may rise higher than its opponent, incline its dorsal region toward the other fish (termed a body roll) and spread its fins, iii)

the mouth may be opened in combination with body roll and fin spreading, iv) a position perpendicular to the other fish's longitudinal axis and above and in front of its head may be combined with spread fins, body roll, open mouth and, occasionally, body quivering. We feel on the basis of our observations that these variations represent increasing levels of display intensity in the order they are listed.

Nipping — One fish bites at another. Variations: i) attempted nip — without physical contact, ii) nip — with contact. Contact was usually with the caudal peduncle or flanks of the recipient but fins and the dorsal regions of the head and body were also nipped. Nipped fins were not held. Scales were occasionally dislodged by nips.

Whirlabout — A fish receives a nip and turns quickly to nip back while the initial aggressor continues to nip or attempt to nip. The two fish wrap themselves around one another, both with mouths open.

Chase — One fish pursues another rapidly. The pursuer may nip, attempt to nip, or chase with closed mouth.

Flee — One fish swims rapidly away from another, usually with all fins folded. Fleeing was often associated with a chase by another fish, but young fish occasionally fled from a displaying opponent that did not chase them.

Avoidance — One fish moves slowly out of the path of another. Aggressors usually ignored a fish that showed avoidance even when the avoidance terminated a charge or was in response to a charge.

Dominance — Qualitative observations indicated that pairs of goldeye form dominance relationships. We considered a fish dominant if its behavior consistently elicited avoidance or fleeing from another fish (the subordinate).

Quantitative Analysis of Agonistic Behavior

Size, sex, residence, and dominance — When two adult fish (Table 1A) were placed together in an observation tank they usually established a dominance relationship during the first day. Dominance was related to the size, sex, and residence of the fish in a pair (Fig. 1). Body color is included in Fig. 1A to illustrate the normal relationship between behavior patterns of a dominant fish and those of a subordinate. Fish that were rated as dark at the end of an observation showed significantly higher frequencies of charging, nipping, chasing, and approaching than did

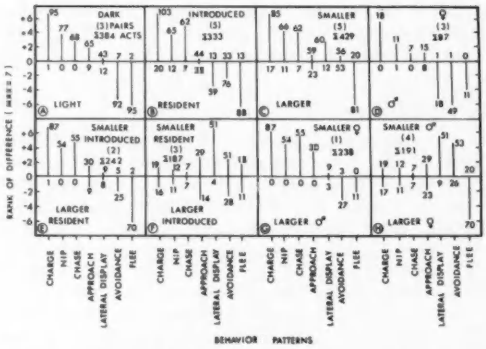

FIG. 1. Ranks of differences in frequency between behaviors performed by adult fish (Table 1A) of different size, sex, and residence. Color indicates fighting success with dominants becoming darker. Ranges of size differences between members of pairs were 0.5–10.5 cm fork length and 19–960 g wet weight. Mean differences were 4.1 cm and 314.2 g. Actual nips were not observed between heterosexual pairs; attempted nips account for the differences illustrated in (D). Data from pairing with animals of the same sex are included in (G) and (H). In (F) and (H), + scores were assigned to the group with the highest frequency of charges. All differences significant ($P > 0.01$, χ^2) except those straddling zero. Numerals in parentheses, numbers of pairs; numerals without parentheses, number of acts.

fish that remained light ($P = 0.01$). The light fish avoided and fled more frequently.

Introduced fish were dominant over resident fish (Fig. 1B), smaller adult fish usually dominated larger adult fish (Fig. 1C), and females tended to dominate males (Fig. 1D). Small, introduced adults were dominant over larger, resident fish (Fig. 1E) while there was little difference between small residents and large introduced fish (Fig. 1F). Smaller females dominated larger fish (Fig. 1G) while there was no clear distinction between larger females and smaller adults of either sex (Fig. 1H). The effects of size, sex, and residency appear to be additive to some extent, and to favor smaller fish, nonresident fish, and females. This conclusion was supported by our qualitative observations.

Responses of adults to the behavior of antagonists — Most agonistic behavior patterns described were exhibited by dominant fish (Fig. 2). Subordinate fish exhibited only four behavior patterns with any consistency: lateral display, approach, avoidance, and fleeing. Avoidance and fleeing often elicited an aggressive response from the dominant fish, particularly during initial interactions. Conversely, any behavior pattern ex-

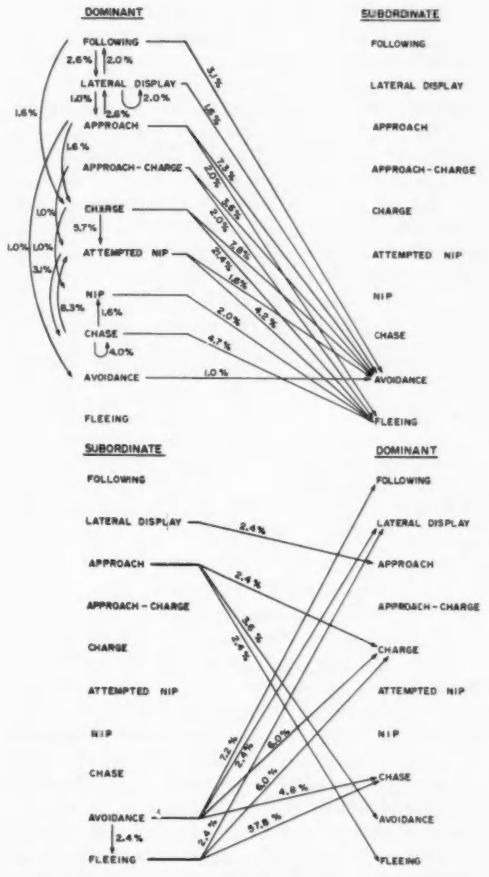

FIG. 2. Percentage of displays by the dominant members of adult pairs (Table 1A) that were followed by displays by the dominant or subordinate (*upper panel*, total 192 acts) and percentages of displays by subordinates that were followed by displays by the dominant fish (*lower panel*, total 83 acts).

hibited by a dominant fish, including avoidance, resulted in avoidance or fleeing by the subordinate fish (Fig. 2). Frequencies of responses of one fish to the behavior of another in a pair were assembled from the same body of observation records used to compile Fig. 1 (Table 1A). We used only responses that occurred more than once, and that followed the initiating pattern within seconds.

Both the dominant and subordinate fish exhibited lateral displays and approaching (Fig. 2). These two behavior patterns were typical of initial agonistic encounters. The goldeye lateral display, like that of juvenile salmonids (Kalleberg 1958; Keenleyside and Yamamoto 1962) occurred when there appeared to be conflicting tendencies, attack and flee. This effect could also be seen in closely matched pairs, which showed large differences in lateral displaying (Fig. 1F and 1H), based on high frequencies of lateral display by one member of each pair.

Once a dominance relationship was established between two individuals, lateral displays, following, approaching, and approach–charge were seldom observed during interactions. The frequency of agonistic interactions declined, and interactions typically consisted of direct attack by the dominant fish. Nearly 30% of the sequences initiated by the dominant fish began with charges. There was also a considerable amount of avoidance exhibited by the subordinate fish once the hierarchy had been established, even as a response to a nonaggressive act by the dominant fish. Fleeing by the subordinate, however, was the most common response to an aggressive act by the dominant fish. In turn, fleeing by the subordinate led to a chase by the dominant in nearly 60% of the cases.

Agonistic behavior of ripe fish — The agonistic behavior of ripe pairs (Table 1B) differed quantitatively from that of nonripe adults.

Ripe heterosexual pairs exhibited a significantly greater percentage of approaches ($P = 0.01$) and significantly lower percentages of charges ($P = 0.01$) and lateral displays ($P = 0.02$) during agonistic encounters when compared with nonripe pairs.

Compared to nonripe males, nonripe females exhibited a higher frequency of all recorded agonistic behavior patterns except lateral display, avoidance, and fleeing (Fig. 1D). When agonistic behavior of ripe males and females was compared, there were no significant differences between the frequencies of approach–charge, attempted nip, nip, or chase. Four behavior patterns did differ significantly (all at the $P = 0.001$ level): ripe males exhibited a higher percentage of lateral displays, avoidance, and fleeing while ripe females approached more frequently.

Behavior sequences — Underyearling fish showed less complex patterns of organization in their agonistic behavior than did adults (Fig. 3). The most common behavior of age 0 fish (Table 1C) was charging followed by nipping, with the most frequent sequence being charge → nip. Avoidance occurred frequently and was the typical response to aggression. Adult fish (Table 1A) performed three main sequences: Charge → chase → nip, avoid → flee, and approach → lateral

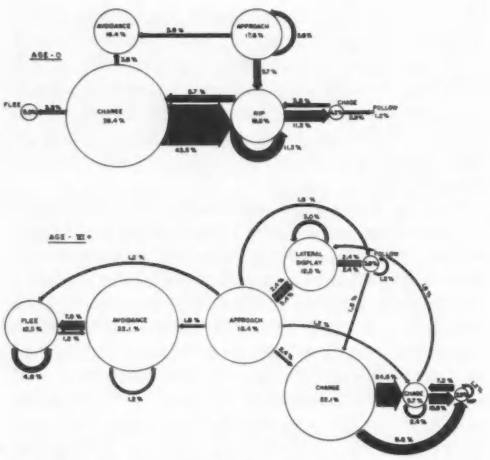

FIG. 3. Frequencies of behavior patterns and the percentage of times they were followed by the performance of other patterns by the same fish. Circle diameter is directly proportional to the frequency of behavior patterns and includes events that did not occur in sequence. Arrow width is directly proportional to the frequency of a sequence.

display → follow. The avoid → flee sequence was typical of subordinate fish; approaching was part of this sequence only during initial agonistic encounters. The approach → lateral display → follow sequence was also characteristic of initial encounters and was exhibited by dominant fish. The charge → chase → nip sequence was typically evident after the establishment of a hierarchy. The behavior of underyearlings emphasized direct attack while adults showed more display and less tendency to charge and nip.

Comparison of frequency of agonistic behavior in three age-groups — Age 0 fish charged, approached, and nipped more frequently than age I fish which, in turn, performed these three behaviors more frequently than did adult fish (Fig. 4). On the other hand, approach–charges and lateral displays were observed only in adult fish. Age 0 and I fish often approached or charged other fish without apparent provocation, then returned immediately to their previous position in the tank. Dominance hierarchies were not observed, and both fish in a pair typically remained aggressive for the duration of observation (up to 12.5 days for age 0 fish). Adult fish often displayed before attacking and interactions were typically long, involving several behaviors. Once dominance was established, usually within the first day, the number of interactions was reduced.

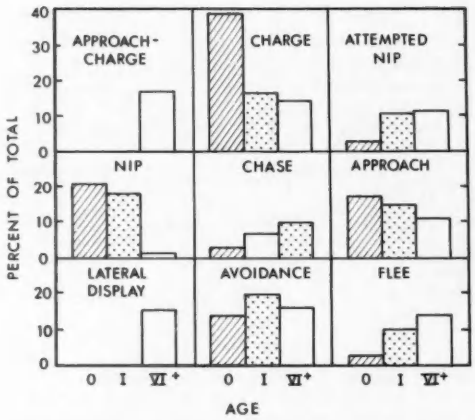

FIG. 4. Comparison of the relative frequencies of agonistic behavior patterns exhibited by age 0, I, and VI–VIII fish. Results for individual behavior patterns are presented as a percentage of the total agonistic behavior exhibited by each age-class.

Spatial distribution of adults — Two adult fish in 3½- or 18-kl tanks tended to separate into two different regions of the tank after the 1st day of contact. Agonistic interactions usually occurred along a boundary between these regions. If a territory is defined as a "defended area," then captive goldeye in large tanks can properly be considered territorial, even though prior residence in a tank does not confer advantage in paired encounters. Goldeye did not occupy a particular station in their territory but moved continuously throughout the defended space. The region defended was found to shift gradually day by day around the tank, although the size of the territory remained about the same.

Comparison of pairs and trios — Minor differences occurred between the behavior of pairs and trios of age 0 fish. Two pairs (Table 1C) performed 52 charges and 4 flees during 20 observations compared to 19 charges and 27 flees in the same time by two trios (Table 1F). In contrast, adults showed a striking difference in behavior with the addition of a third fish in a 3.5-kl tank. The frequency of agonistic behavior was drastically reduced — five pairs (Table 1A) performed 422 agonistic acts during four observations per pair while five trios (Table 1G) performed only 33 agonistic acts during the same period. This difference corresponded with a qualitative change in behavior, as pairs divided the tank into two territories while trios tended to school.

Discussion

Fighting may play some part in the reproduction of adult goldeye, but this is probably not its primary function, since juveniles and nonbreeding adults fight more vigorously than do ripe adults. We feel that goldeye agonistic behavior probably functions as an ecological spacing mechanism, distributing goldeye throughout available habitat. Another member of the order Osteoglossiformes, the arawana (*Osteoglossum bicirrhosum*) exhibits intense agonistic behavior, in which considerable physical damage may be inflicted (Takeshita 1973). Young arawana, like goldeye, fight more often than adults. Nonreproductive territoriality has also been described in centrarchids and salmonids (Greenberg 1947; Hoar 1951; Fabricius and Gustafson 1955; Lindroth 1956; Chapman and Bjornn 1969; Jenkins 1969). Its function is often regarded as being the distribution of conspecifics to promote efficient utilization of food resources.

Data from our collecting trips support the hypothesis that goldeye are dispersed during the summer. Adult fish were never caught in close proximity when nets were run every hour, with a few exceptions when the fish were migrating. Underyearlings were also dispersed and became gregarious during the fall migration. Fernet (1973) estimated their density in the Peace–Athabasca Delta to be one fish per 50 m^2. Dispersal could result from territoriality or the maintenance of individual distance. Goldeye typically inhabit turbid water, where visibility may be less than one body length. Under these circumstances they may simply attack any conspecific which comes close enough to be seen, a situation which fits Hediger's (1955) definition of individual distance.

Both quantitative analysis of fighting success and qualitative observation indicate females are more aggressive than males, smaller fish are more aggressive than larger ones, and introduced fish are more aggressive than resident fish. These conclusions run counter to the usually accepted relationships between fighting success and sex, size, and residency in fish (Frey and Miller 1972).

We speculate that this unusual situation may be explained by postulating a relationship between feeding and agonistic behavior. Females have a faster growth rate than males and produce many eggs while the males produce little milt (Kennedy and Sprules 1967). Females will therefore have higher metabolic demands than males and may be more highly motivated to acquire and defend feeding territories. Smaller goldeye may also have greater metabolic demands than large fish since growth increments decline with age and size–metabolism relationships would favor a higher metabolic rate in small fish. The decline in overt aggression with age (and therefore size) is probably also related to the increase in ritualization which occurs in the development of goldeye agonistic behavior. Resident fish did not defend their territories vigorously in our laboratory. We did not feed the fish in the observation tanks. If defense of a feeding territory depends on the quality or quantity of food present, then our tanks may not have been worth defending. Magnuson (1962) found that larger medaka (*Oryzias latipes*) were more aggressive when food was available in limited quantities than when it was absent altogether. In summary, classes of goldeye with high metabolic requirements win agonistic encounters; unfed resident fish lose to introduced fish. It would be particularly desirable to observe the effects of feeding on the resident–introduced relationship. Unfortunately it is difficult to persuade goldeye to feed in captivity.

Increasing the number of fish per tank was observed to subdue aggressive behavior in adult goldeye. A similar effect was found in wild juvenile Atlantic salmon (*Salmo salar*) by Keenleyside and Yamamoto (1962) and Fenderson and Carpenter (1971), and in the ayu (*Plecoglossus altivelis*) by Kawanabe (1969). The effect of gradually increasing the number of young Atlantic salmon resulted in increased aggressive responses until a threshold was reached, at which point the frequency of agonistic interactions rapidly declined. Salmon not holding territories formed tight schools in the observation tanks, while the dominant fish continued to hold territories on the substrate. The results of increasing goldeye densities was the immediate loss of territory by all fish, followed by a schooling response. The threshold for this effect was very low (three fish in a 3.5-kl tank). Captive goldeye hold open-water territories, rather than substrate-based territories like those of Atlantic salmon. When increased density does occur, there is no place in the confines of an observation tank for nonterritory-holders to retreat. This may cause the schooling response, and the decrease in the frequency of aggressive interactions.

Sexual Behavior

Ripe fish were in general less aggressive than sexually regressed adults. The only behavior pattern that ripe pairs exhibited with a significantly higher frequency than nonripe pairs was approaching, performed mainly by ripe females.

This may indicate the approach is an initial female pattern during sexual behavior. Ripe males continued to exhibit a high frequency of lateral displays, as did nonripe males; this may be one of the initial male behavior patterns during spawning. An alternative hypothesis is that ripe males did not interpret the approach as the initiation of sexual behavior, but rather an aggressive act, and responded with the lateral display. Study of the behavior of breeding fish in captivity or in the field would no doubt clarify the relations between agonistic and reproductive behavior.

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Experimental Confirmation of the Olfactory Hypothesis with Homing, Artificially Imprinted Coho Salmon (*Oncorhynchus kisutch*)

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To test the olfactory hypothesis of salmon homing, fingerling coho salmon (*Oncorhynchus kisutch*) were exposed to low concentrations of an odorous synthetic chemical, morpholine, during presmolt and smolting periods. Equal numbers of these fish were not exposed (controls). Both groups were stocked directly into Lake Michigan near Oak Creek, South Milwaukee, Wisconsin. Another paired group of exposed and unexposed fish was released 13 km north of Oak Creek. During the adult spawning migration 18 mo later, morpholine was dripped into Oak Creek and the returning salmon were censused.

For four experiments over 2 consecutive yr, the number of exposed and unexposed fish captured at the artificially scented stream were, 216 vs. 28, 437 vs. 49, 647 vs. 65, and 439 vs. 55. These differences were highly significant ($P < .001$).

During the 3rd yr, morpholine was not added to the stream during the spawning migration and exposed and nonexposed fish returned in equal, low numbers (51 vs. 55, $P > .05$). These results confirm the existence of odor imprinting and long-term olfactory memory in coho salmon. It is possible that this mechanism is also used in the natural imprinting situation.

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Dans le but de vérifier l'hypothèse olfactive du retour à la rivière natale chez le saumon, des alevins de saumon coho (*Oncorhynchus kisutch*) de la grosseur du doigt ont été exposés à de faibles concentrations d'une substance chimique odoriférante, la morpholine, avant et durant la smoltification. Des nombres égaux de ces poissons ne furent pas exposés (témoins). Les deux groupes ont été relâchés directement dans le lac Michigan, près du ruisseau Oak, Milwaukee-Sud, Wisconsin. Un autre groupe apparié de poissons exposés et non exposés a été relâché à 13 km au nord du ruisseau Oak. Au moment de la migration reproductrice des adultes, 18 mois plus tard, nous avons fait dégoutter de la morpholine dans le ruisseau Oak et compté les saumons qui y montèrent.

Dans quatre expériences couvrant 2 a. consécutives, les nombres de poissons exposés et non exposés capturés dans le cours d'eau parfumé artificiellement furent de 216 *vs* 28, 437 *vs* 49, 647 *vs* 65 et 439 *vs* 55. Ces différences sont hautement significatives ($P. < .001$).

A la 3[e] a., nous n'avons pas ajouté de morpholine au cours d'eau durant la migration reproductrice, et les poissons exposés et non exposés retournèrent au cours d'eau en nombres égaux et faibles (51 *vs* 55, $P > .05$). Ces résultats confirment l'existence de l'impression d'une odeur et d'une mémoire olfactive à long terme chez le saumon coho. Il est possible que ce mécanisme serve également dans le cas d'une impression naturelle.

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SALMONIDS return from the open waters of lakes or oceans with great specificity to their home-stream to spawn (Hasler 1966; Harden-Jones 1968; Stasko 1971). The role of olfaction in the stream part of these migrations has been proposed. Many laboratory experiments have demonstrated the extreme acuity of the olfactory system in fish (Teichmann 1962; Hasler 1966; Kleerekoper 1969) and that fish can distinguish between the odors of natural waters (Hasler and Wisby 1951; McBride et al. 1964; Walker 1967). Experimental studies support the theory that the soils and vegetation of a drainage basin provide

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a unique cue for each stream (Hasler 1966). Sensory impairment experiments have indicated that the intact olfactory sense was necessary for correct homing to occur (Stasko 1971). However, the effects of sensory impairment operations are not known in the latter experiments (Harden-Hones 1968).

Transplantation studies indirectly support the olfactory hypothesis. Fingerling salmon taken from their homestream or hatchery shortly before the beginning of their downstream migration and transplanted to a second stream returned to the second stream to spawn (Carlin 1959, 1968; Donaldson and Allen 1957; Peck 1970; Jensen and Duncan 1971; Ricker 1972). Two important conclusions can be drawn from these results. First, they have demonstrated that the memory of the characteristics of the homestream are not inherited. Second, they have indicated that there exists a rapid learning process at the time the juvenile salmon begin their downstream migration.

The studies mentioned above have not demonstrated odor imprinting and a long-term olfactory "memory" of the homestream, nor given a final proof of the theory. Therefore, Hasler and Wisby (1951) proposed a crucial experiment to test the olfactory hypothesis, namely to imprint salmon fingerlings in a hatchery to a synthetic odor (used in place of natural odors) and attempt to decoy them to a simulated homestream scented with this odor during the spawning migration. The objective of this study was to perform this experiment. Preliminary findings of these studies were reported earlier (Madison et al. 1973; Scholz et al. 1973, 1975; Cooper and Hasler 1973, 1974, 1976; Cooper 1974).

Materials and Methods

Coho salmon fingerlings, *Oncorhynchus kisutch*, were exposed to morpholine during their presmolt and smolting period at the Wild Rose Fish Hatchery or at the South Milwaukee Water Filtration Plant. An equal number of fish were not exposed (controls). Both groups were marked by finclipping, held during the 34- to 46-day experimental period, and then released directly into Lake Michigan at sites near Oak Creek or 13 km north of Oak Creek. During the spawning migration 18 mo later, morpholine was dripped into Oak Creek to simulate a homestream for the exposed fish. The number of morpholine exposed and unexposed salmon returning to the river was censused by several methods including creel survey, electrofishing, and gillnetting.

Salmon with similar genetic backgrounds (eggs from Lake Michigan fish taken from one location) were used in all paired groups of exposed and unexposed salmon. Both groups were marked with symmetrical finclips whenever possible, before the start of the imprinting experiments. It was thus possible to monitor mortality owing to finclipping, handling, and other causes during the experimental period. There were no reports of differential mortality owing to different finclips at Wisconsin hatcheries, nor did we observe an unequal number of dead fish in the paired imprinted and nonimprinted groups.

Morpholine (diethylenimide oxide) was metered into the experimental raceways to give a concentration of 5×10^{-5} mg/liter in the water. Four-channel parastalic pumps were used to deliver stock solutions of the chemical below the water discharge to insure rapid and complete mixing through the raceway. Fish in the control raceways or tanks were not exposed to morpholine. It was not possible for the morpholine-scented water to mix with the water in the control tanks.

In most experiments, salmon were exposed for approximately 3 wk before until 2 wk after the first signs of smolting behavior.

	Exposure period for imprinting		
Experiment	Start	End	Total days
1	April 13, 1971	May 16	34
2a, 2b, 2c	April 3, 1972	May 18	46
3a, 3b	April 1, 1973	May 14	44
3c	May 12, 1973	May 14	2

Morpholine was selected for imprinting because 1) it is not known to occur in natural waters, 2) it is stable in the natural environment, 3) it is infinitely soluble in water, 4) it is an organic compound, 5) it could be detected by coho salmon at low thresholds, and 6) it is not an attractant for naive salmon. Earlier work (Hasler and Wisby 1951; Idler et al. 1961; Cooper et al. 1974) indicated that natural olfactory substances were organic. Wisby (1952) and Cooper and Hasler (1973) demonstrated that coho salmon have thresholds of around 10^{-8} mg/liter of morpholine.

The same water source, artesian spring water, or alternately Lake Michigan water, was used to supply raceways containing paired groups of salmon (Tables 1 and 2). These water sources were considered "neutral" because they were not connected with any river draining into Lake Michigan and, therefore, could not provide homing fish with any sensory information about the location of a river.

After smolting, both groups of fish were trucked to Lake Michigan and stocked near the mouth of Oak Creek at South Milwaukee, Wisconsin, or 13 km north of Oak Creek (Fig. 1). The purpose of stocking the fish directly into Lake Michigan was to eliminate exposure to the future homestream (Oak Creek) as much as possible. Since there was no downstream migration, the possibility that fish would learn alternative sensory cues from the river or stream was reduced.

Spawning Season

During the fall spawning migration, 18 mo after smolt release, morpholine was metered into Oak Creek to give the creek water approximately the

TABLE 1. History of coho salmon (*Oncorhynchus kisutch*) caught at Oak Creek 1971–74. Locations are shown on Fig. 1. Morpholine was used as the imprinting chemical.

Experiment	Finclip[a]	Treatment	Hatchery source	Site of imprinting	Release	Decoy site
1	D RP-Ad RV-LV	Imprinted Imprinted Control	Hayward	South Milwaukee Water Filtration Plant	Oak Creek	Oak Creek
2a	RM LM	Imprinted Control	Wild Rose	South Milwaukee Water Filtration Plant	Oak Creek	Oak Creek
2b	RP-Ad LP-Ad	Imprinted Control	Wild Rose	Wild Rose Fish Hatchery	Milwaukee Breakwater	Oak Creek
2c	LV-Ad RV	Imprinted Control	Wild Rose	Wild Rose Fish Hatchery	Oak Creek	Oak Creek
3a	RP-Ad LP-Ad	Imprinted Control	Crystal Springs	South Milwaukee Water Filtration Plant	Oak Creek	No morpholine added

[a]Finclips are: RP = right pectoral, LP = left pectoral, RV = right ventral, LV = left ventral, Ad = adipose, D = dorsal, RM = right maxillary, LM = left maxillary.

TABLE 2. Census record of salmon caught at Oak Creek 1971–74. χ^2 test used to test for significance.

Experiment	Treatment	Morpholine concn	No. released	No. jacks recovered and %	No. adults recovered and %	Total no. recovered and %	*P*
				1971	1972		
1	Imprinted	1×10^{-4}	4,000	4 (0.10)	103 (2.58)	107 (2.68)	
	Imprinted	1×10^{-5}	4,000	27 (0.68)	82 (2.02)	109 (2.70)	< .001
	Control	None	8,000	3 (0.04)	25 (0.31)	28 (0.35)	
				1972	1973		
2a	Imprinted	5×10^{-5}	5,000	4 (0.08)	433 (8.66)	437 (8.74)	< .001
	Control	None	5,000	3 (0.06)	46 (0.89)	49 (0.95)	
2b	Imprinted	5×10^{-5}	8,200	20 (0.24)	627 (7.65)	647 (7.89)	< .001
	Control	None	10,000	1 (0.01)	64 (0.64)	65 (0.65)	
2c	Imprinted	5×10^{-5}	5,000	16 (0.32)	423 (8.46)	439 (8.78)	< .001
	Control	None	5,000	2 (0.04)	53 (1.06)	55 (1.10)	
					1974		
3a	Imprinted	5×10^{-5}	5,000	(A)	51 (1.02)	51 (1.02)	> .10
	Control	None	5,000		55 (1.10)	55 (1.10)	

(A) See Table 3.

FIG. 1. South Milwaukee area and the State of Wisconsin hatcheries. 1, the water intake for the tanks at the South Milwaukee Water Filtration Plant; 2, the Oak Creek stocking site; and 3, the Milwaukee stocking site.

same concentration as used for imprinting. Because of the variable flow rate of the creek and the constant rate of morpholine addition to the stream, the actual stream concentration probably varied but rarely exceeded the range of 3×10^{-4} to 1×10^{-5} mg/liter. Near the discharge of chemicals into the creek morpholine might have reached concentrations as high as 1×10^{-3} mg/liter.

Returning fish were captured in Oak Creek by electrofishing; gillnets were also used occasionally. Creel surveys were taken from anglers along the creek from 3 to 7 times per day throughout the season; hence the census effort tended to be uniform. Census clerks recorded information on number of fish, finclips, location of capture, time of day, general weather conditions, and total number of anglers in a fishing location.

Additional methods are given in Table 1.

Results

Exposure to morpholine during presmolt and smolting periods is sufficient for imprinting coho salmon to this chemical so that they can use this information to locate a stream scented with it. In experiments 1, 2a, and 2c, 216 vs. 28, 437 vs. 49, and 439 vs. 55 exposed and unexposed fish, respectively, homed to an artificially scented stream (Table 2). These differences are highly significant. The concentrations of morpholine used for imprinting in these experiments was sufficient, with no significant difference in the effectiveness of imprinting occurring, whether a concentration of 1×10^{-5}, 1×10^{-4}, or 5×10^{-5} mg/liter was used. The "neutral" hatchery water used for experiment 2c did not significantly affect the outcome of the experiment compared to experiments 1 or 2a.

The smolt release location need not be in the vicinity of the future (simulated) homestream

for successful homing of the imprinted fish to occur. Instead of being released at Oak Creek, the smolts in experiment 2b were released 13 km north of Oak Creek at the Coast Guard station near Milwaukee Harbor (Fig. 1). The recovery of 647 experimentals and 65 controls was similar to the recovery of fish in experiments 2a and 2c, indicating the smolt release site can be at least 13 km from the future homestream.

All groups of fish released in the 2nd yr returned in a roughly normal distribution with the peak of the run occurring on November 4 (Fig. 2). There was a striking correlation in the timing of all groups, although fish in experiment 2a, 2b, and 2c differed in imprinting or release locations. Note also that imprinted and nonimprinted fish showed similar catch distributions.

Significant differences in returns of imprinted vs. nonimprinted fish to Oak Creek occurred only when morpholine was present. In the fall of 1974 morpholine was not added to the stream during the adult salmon run. In experiment 3a, 51 exposed salmon compared to 55 controls were captured. There was no significant difference between the returns of these two groups.

Imprinting to morpholine also occurs when a concentration higher than 5×10^{-5} is used, but was no more effective than imprinting at lower concentrations. In this experiment (3b), salmon were exposed to concentrations of 1×10^{-2} mg/liter morpholine. Seventeen of these imprinted jacks (precocious juveniles) were captured in the fall of 1973 when morpholine was being added into Oak Creek, whereas only 3 control fish (experiment 3a, Table 3) were captured. This suggests that imprinting does occur at high concentrations but is no more effective than 5×10^{-5} mg/liter (19 imprinted jacks returned in experiment 3a). Lower numbers of adults (29) returned in 1974 when no morpholine was added to Oak Creek.

Very short periods of morpholine exposure seem sufficient to imprint fish successfully. In experiment 3c, a group of fish was imprinted only during the last 2 days before release at 5×10^{-5} mg/liter (May 12–14). Twenty-seven imprinted jacks and only three controls were captured in 1973 when morpholine was in the creek. Fifty-one adults were captured in the fall of 1974 with no morpholine in Oak Creek. This compares with 51 adults captured in Experiment 3a.

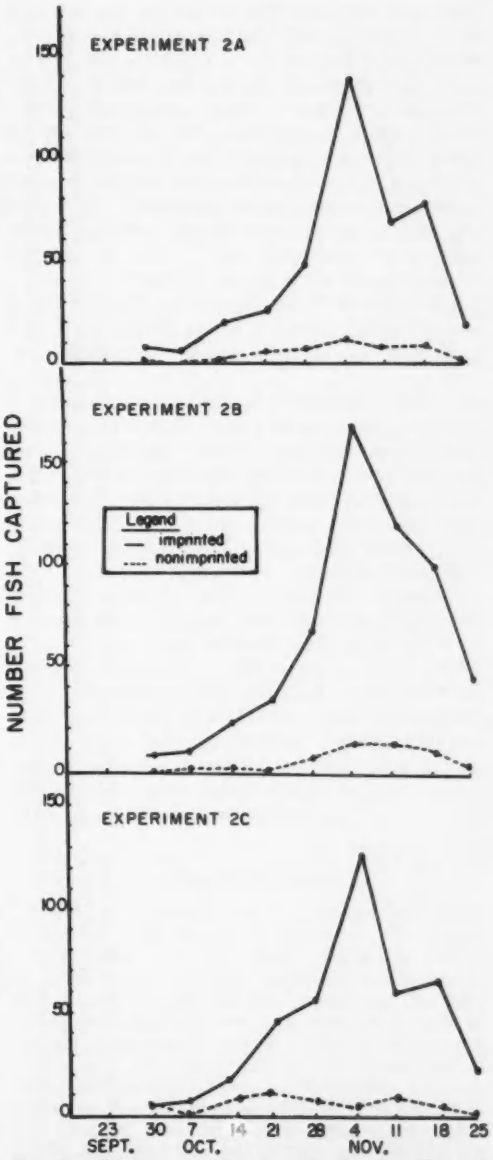

FIG. 2. Catch curves for experiments 2a, 2b, and 2c. Although fish in each experiment differed in stocking location or imprinting site, there is a striking correlation in the peak of each run (November 4). Note that imprinted and nonimprinted fish show the same time distribution.

Discussion

In four series of experiments over a period of 3 yr it was shown that salmon imprint to morpholine and, after 18 mo, use this odor to locate a simulated homestream. Further, it was possible to decoy these fish into the morpholine-scented stream even when smolts are released from a distance of 13 km. While further experiments need to be performed, imprinting was shown to

TABLE 3. Census record of salmon caught at Oak Creek, South Milwaukee, 1973, 1974. All salmon had been obtained from and imprinted at the Crystal Springs Fish Hatchery in 1973. 5,000 fish were released in each group. Note: Morpholine was released into Oak Creek in 1973, but not in 1974. See Table 1 for finclips. χ^2 test used to test for significance.

Experiment	Finclip	Odor treatment	Exposure time (days)	Concn of odor (*mg/liter*)	Release location	1973 No. jacks recovered	1974 No. adults recaptured	*P*
3a	LP-Ad	None	—	—	Oak Creek	3	55	> .10
	RP-Ad	Morpholine	44	5×10^{-5}	Oak Creek	19	51	
3b	LV-Ad	Morpholine	44	1×10^{-2}	Oak Creek	17	29	< .05
3c	RV-Ad	Morpholine	2	5×10^{-5}	Oak Creek	27	51	< .005

occur within 2 days of chemical exposure near the end of the smolting period. In addition, morpholine at concentrations as low as 1×10^{-5} mg/liter appeared to be sufficient for imprinting. Higher concentrations did not appear to increase homing abilities of the imprinted fish.

Smolting appears to be a developmental phase in the life cycle of salmon, during which they can learn cues about the stream to which they return. Experiments with transplanted fish suggest that the smolting period of coho salmon would be the most appropriate time to attempt artificial imprinting. In these experiments, 64 days of exposure (Donaldson and Allen 1957) for coho salmon and 2 days (Carlin 1959, 1968) for Atlantic salmon (*Salmo salar*) to a stream water during smolting was sufficient to have homing to that stream. Similarly, Jensen and Duncan (1971) found with coho salmon that 2 days of exposure to spring water during the smolting period was enough time to allow fish to return to the same water. In contrast, Peck (1970) released coho salmon that had completed smolting into a river flowing into Lake Superior and found a large amount of straying to other streams.

The term "imprinting" in connection with olfaction is used to refer to the process of learning the odors of the homestream (perhaps irreversibly) during some "critical" or "sensitive" period in the early life of the salmon. There must be retention of this odor information, with no subsequent reexposure until sexual maturity and the spawning migration. This definition has been broadened from the classic definition to include environmental variables as suggested previously (Thorpe 1963; Hess 1973).

The "critical" period, in our context, specifies the time in the life of the juvenile salmon during which the odors of the parent stream are indelibly learned. This period may be said to be critical because if the memory is not formed before the fish leaves the homestream, then these odor characteristics will never again be available for learning. Because there is some variability and perhaps flexibility in the time of imprinting, we designate it as the "sensitive" period, specifying a period during which the salmon can learn odor cues unique to the homestream. Therefore, we feel that Hess (1973) is too restrictive in not accepting the use of "imprinting" until a critical, as opposed to sensitive, period is demonstrated.

The relation between artificial imprinting to a single odor and imprinting to perhaps multiple odors in the natural environment is not known. The possibility of a redundancy in the natural system in which more than one odor is imprinted cannot be discounted.

The background water used for holding the fish did not appear to influence the imprinting process in our experiment. The numbers of imprinted fish returning to the scented stream were similar for fish held in Lake Michigan, Wild Rose, and Crystal Springs hatchery water. Further work on the influence of a background water from a natural source on artificial imprinting is presently being conducted at our laboratory. The possibility of an odor dominance by the natural odors needs to be explored. The effectiveness of artificial imprinting in natural waters must also be assayed.

Further experiments must be completed in order to select additional chemicals for imprinting. Experiments are in progress (including one with phenethyl alcohol used as an imprinting chemical).

Behavioral and electrophysiological experiments support and amplify many of the results reported here. Significantly higher amplitude responses in electroencephalographic recordings were observed from imprinted compared to nonimprinted salmon when tested with water samples containing morpholine (Cooper 1974; Cooper and Hasler 1973, 1974, 1976). Similarly, displaced imprinted salmon tracked with ultrasonic transmitters stopped at a morpholine-scented area other than Oak Creek. Nonimprinted fish did not stop (Madison et al. 1973; Scholz et al. 1973, 1975).

In conclusion, results from 3 yr of experiments with morpholine indicate that coho salmon can learn, retain, and use artificial chemical information to achieve successful homing. Salmon possessing this ability would likely use this mechanism in the natural environment.

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Infection of Lumpfish (*Cyclopterus lumpus*) with Larvae and of Atlantic cod (*Gadus morhua*) with Adults of the Copepod, *Lernaeocera branchialis*, in and adjacent to the Newfoundland area, and Inferences Therefrom on Inshore–Offshore Migrations of Cod

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TEMPLEMAN, W., V. M. HODDER, AND A. M. FLEMING. 1976. Infection of lumpfish (*Cyclopterus lumpus*) with larvae and of Atlantic cod (*Gadus morhua*) with adults of the copepod, *Lernaeocera branchialis*, in and adjacent to the Newfoundland area, and inferences therefrom on inshore–offshore migrations of cod. J. Fish. Res. Board Can. 33: 711–731.

The lumpfish, *Cyclopterus lumpus*, was found to be a common intermediate host of the larvae of the copepod, *Lernaeocera branchialis*, in the Newfoundland and neighboring areas. Large numbers of these larvae occurred on the gills of lumpfish from inshore Newfoundland areas from the latter half of June to the first half of August, whereas only minor infection was found in any month in offshore areas. Some larvae of the year reached the final or seventh stage on the intermediate host in May on the west coast of Newfoundland and in June on the east coast. By July on the west coast and early August on the east coast, this was by far the most numerous stage present. The larvae were attached mainly near the tips of the gill filaments. Most larvae were attached to the gills of the first two branchial arches, less to those of the third, and much less to those of the fourth. There were more larvae on the right than on the left gills. Infection of the final host Atlantic cod (*Gadus morhua*), by stage-7 female *L. branchialis* was apparently mainly inshore.

Adult *L. branchialis* on Atlantic cod, typically located near the anterior ventral apex of the branchial arches, were numerous throughout the year in the areas of high infection. Infection rates of cod with the adult parasite were usually considerably higher near the coast than farther offshore, the rates declining with distance from the coast. Rates of infection with the adult parasite usually increased from the smaller to intermediate cod lengths and declined rapidly at greater lengths. Infection rates were found useful as evidence of inshore and offshore migrations of cod. Infection with the copepod apparently delayed sexual maturity in cod. Most infected cod (86%) had one adult copepod and declining numbers (12–0.05%) possessed two to five adult copepods. The Greenland cod, *G. ogac*, was the only other fish of the area found to be highly infected with adult *L. branchialis*.

TEMPLEMAN, W., V. M. HODDER, AND A. M. FLEMING. 1976. Infection of lumpfish (*Cyclopterus lumpus*) with larvae and of Atlantic cod (*Gadus morhua*) with adults of the copepod, *Lernaeocera branchialis*, in and adjacent to the Newfoundland area, and inferences therefrom on inshore–offshore migrations of cod. J. Fish. Res. Board Can. 33: 711–731.

On constate que la grosse poule de mer, *Cyclopterus lumpus*, est un hôte commun des larves du copépode *Lernaeocera branchialis* à Terre-Neuve et dans les régions avoisinantes. Ces larves se trouvent en grand nombre sur les branchies de la poule de mer des régions côtières de Terre-Neuve de la seconde moitié de juin à la première moitié d'août. Par ailleurs, on n'observe que des infestations mineures en aucun temps dans les eaux du large. Quelques larves de l'année

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atteignent le septième ou stade larvaire final en mai sur la côte occidentale et en juin sur la côte orientale de Terre-Neuve. En juillet sur la côte occidentale et en août sur la côte orientale, ce stade est de beaucoup le plus abondant. Les larves sont surtout fixées sur l'extrémité des filaments branchiaux. La plupart des larves se trouvent sur les deux premières branchies, moins sur la troisième et encore moins sur la quatrième. Elles sont plus nombreuses sur les branchies de droite que sur celles de gauche. L'infestation de l'hôte final, la morue franche, *Gadus morhua*, par les femelles au stade 7 de *L. branchialis* se produit apparemment surtout près de la côte.

Les *L. branchialis* adultes de la morue franche, typiquement placés près du sommet ventral antérieur des arcs branchiaux, sont présents en grand nombre à l'année longue dans les régions de forte infestation. Les taux d'infestation de la morue par le parasite adulte sont ordinairement beaucoup plus élevés près de la côte que plus au large, l'infestation diminuant en fonction de la distance de la côte. L'infestation de la morue semble se trouver surtout près de la côte. Les taux d'infestation augmentent ordinairement des petites morues aux morues de taille intermédiaire et diminuent rapidement chez les morues plus grandes. Ces taux sont des indicateurs utiles des migrations de la morue vers la côte ou vers le large. L'infestation par le copépode semble retarder la maturité sexuelle de la morue. La plupart des morues infestées (86%) possèdent un copépode adulte, et des nombres graduellement moindres (12–0.05%) ont de deux à cinq copépodes adultes. Le saida, *G. ogac*, est le seul autre poisson de la région qui soit fortement infesté par *L. branchialis* adulte.

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In this paper we present the results of researches on infection in its intermediate and final hosts in the northwest Atlantic by the parasitic copepod *Lernaeocera branchialis* (Linnaeus).

In the North Sea, the intermediate host of *L. branchialis* is usually the flounder, *Platichthys flesus*, at the Faroe Islands, *Microstomus kitt*, and in the White Sea, *P. flesus*, *Liopsetta glacialis*, and *Cyclopterus lumpus* (Kabata 1960). Wilson (1932) and Sproston (1942) stated that the larval forms of *Lernaeocera branchialis* had not yet been found on an intermediate host in the western Atlantic. Scientists at the St. John's, Nfld. Biological Station began their search for an intermediate host of *L. branchialis* in June–August 1949 by field examination of the gills of 145 winter flounder, *Pseudopleuronectes americanus*, taken at Fogo, Dildo, Ramea, and Port-aux-Basques, and the further examination in the laboratory under a dissecting microscope of an additional 48 specimens of winter flounder from these areas, but no larvae of the copepod were found. The winter flounder specimens were taken close to the beaches and were 20–47 cm long. In 1951, for the first time in the western Atlantic, the lumpfish, *C. lumpus*, was found to be host of these larvae. The larvae were first found attached to the gills of lumpfish taken close to the shore at Bonavista on June 18, 1951. No other intermediate host for larval *L. branchialis* has yet been found in the western Atlantic, but no extensive search has been made. Brief reports on some of the preliminary results of these investigations were given in Templeman et al. (1950), Fleming and Templeman (1951), Newfoundland Fisheries Research Station (1952), Templemen (1953), and Templeman and Fleming (1963).

Kabata (1961) concluded, from a comparative study, that the adult *Lernaeocera* from Atlantic cod and its larvae from lumpfish of the northwest Atlantic were identical with the European *L. branchialis*. Sherman and Wise (1961), referring to researches in 1957, found six lumpfish in the Eastport (Maine) area carrying *L. branchialis* larvae on their gills and reported on the frequency of infection of cod with the adult copepod in the waters off northeastern United States. The names of localities and areas referred to in the text are given in Fig. 1.

Materials and Methods

Larval *Lernaeocera branchialis* on Lumpfish Gills

In 1951–70, lumpfish specimens were periodically collected from various coastal and offshore areas adjacent to Newfoundland and Labrador. On the east coast of Newfoundland, the main inshore sampling areas were at Bonavista and St. John's, where the specimens were obtained from fishermen when they returned to port about 1–2 h after hauling their salmon nets and codtraps. Specimens from other inshore areas were collected by field personnel during the course of their trips on small research vessels, using various gear near the shore (spear, gillnet, beach seine, otter trawl). Specimens from the offshore areas were invariably obtained incidentally from bottom otter-trawl sets by research vessels for groundfish. Except for the lumpfish taken in 180–255 m by otter trawl in Fortune and Hermitage bays, all inshore specimens were taken in depths less than 40 m by gear set less than 1 naut. mile from shore. All of the offshore specimens were taken more than 5 naut. miles from the coast. As soon as possible after capture, the set of branchial arches and gills from each lumpfish was carefully removed and placed separately in a bottle containing 10% formalin. The

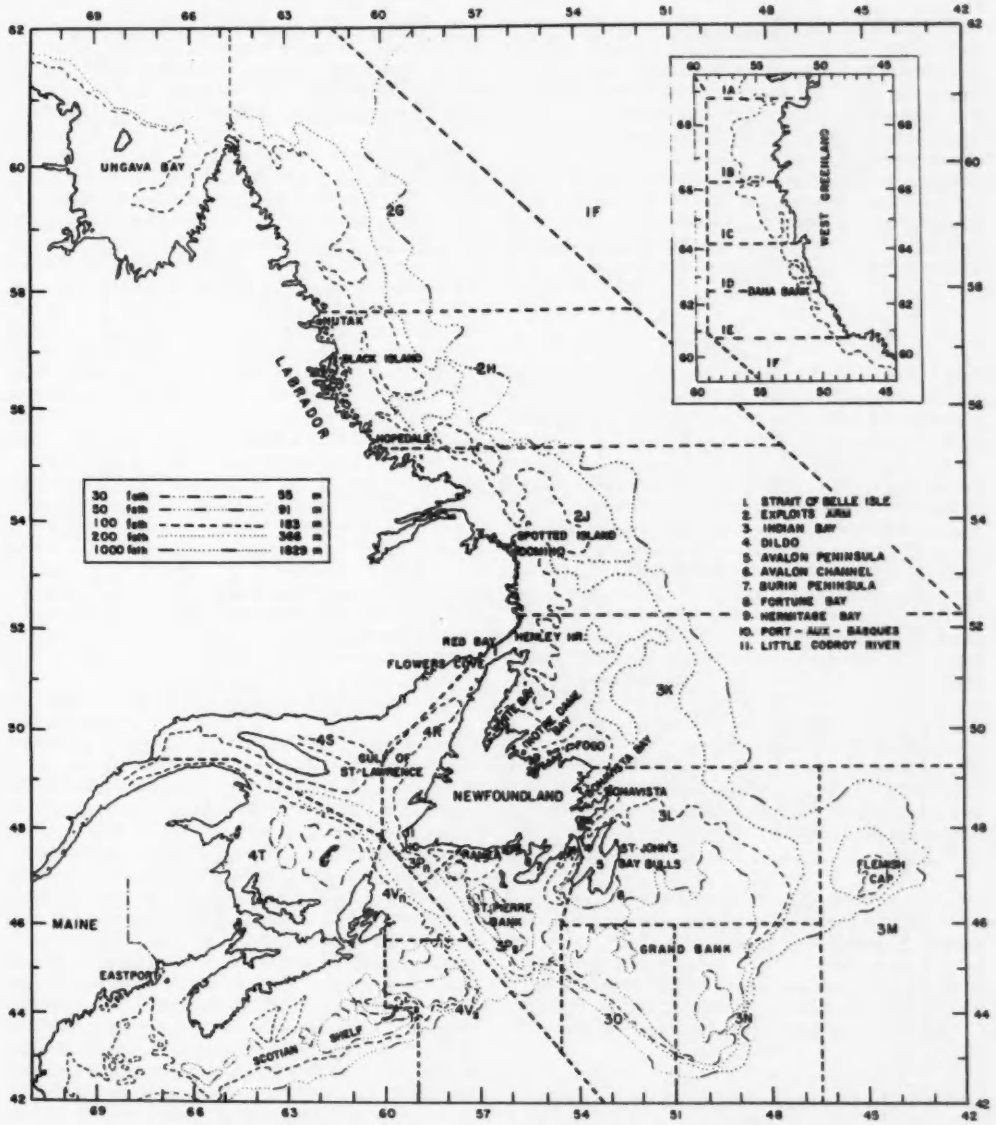

FIG. 1. Localities and ICNAF subareas and divisions mentioned in the paper.

gills of 156 lumpfish from inshore and 178 from offshore Newfoundland areas, and 2 from Dana Bank off West Greenland were examined for *L. branchialis* larvae (Fig. 2).

Of the lumpfish examined from the coastal areas, 112 were taken during May to August, when they migrate to the shallow warm inshore waters to spawn. Consequently most of the inshore specimens were large mature fish. Except for one 17-cm male from Indian Bay (Bonavista Bay) and 10 (10–20 cm) lumpfish from the south coast, all 56 males (26–36 cm) and 78 females (37–52 cm) were mature spawning or spent fish. All 5 specimens taken in August at Flowers Cove were mature males that were speared as they guarded the recently laid eggs in a depth of about 2 m. Of the lumpfish examined from the offshore areas, there were 28 mature males (21–43 cm), 114 mature females (35–53 cm), and

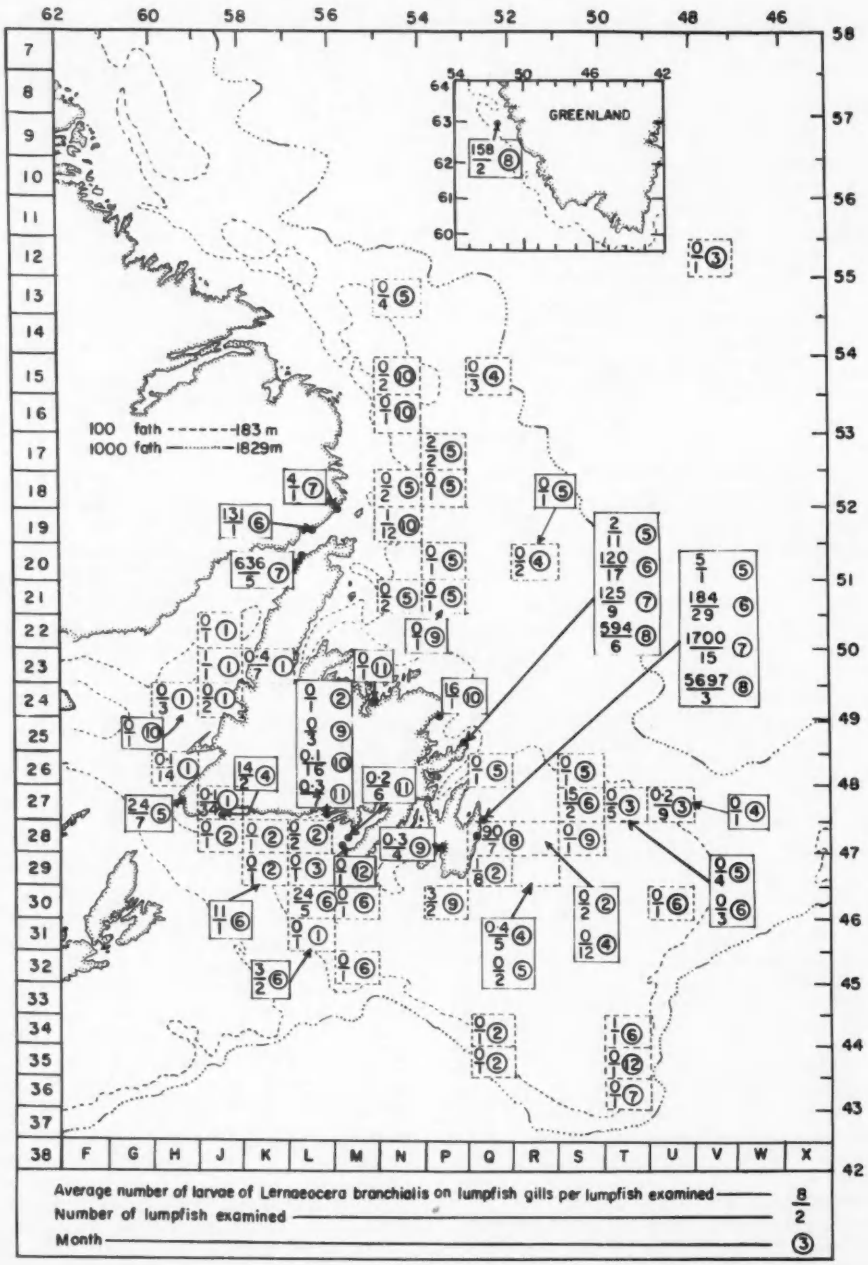
GREENLAND
100 fath ------183 m
1000 fath ——1829m
Average number of larvae of Lernaeocera branchialis on lumpfish gills per lumpfish examined
Number of lumpfish examined
Month

FIG. 2. *Lernaeocera branchialis*: distribution of infection of lumpfish (*Cyclopterus lumpus*) with larvae of the copepod. (The locations of all Newfoundland and Labrador inshore, including south coast bay recaptures, are marked with a black dot. All others were over 5 naut. miles from shore and were considered to be offshore.)

14 females (35–40 cm) that were recorded as immature or maturity condition unknown. The size of the gills of the remaining lumpfish specimens, whose length, sex, and maturity were not recorded, indicated that they were within the size range of the mature fish.

Preparatory to the examination of the lumpfish gills for *L. branchialis* larvae, the 8 branchial arches (1–4 on right and left sides) were carefully separated and placed in water in separate flat translucent dishes, that were appropriately labeled R1, R2, R3, R4, L1, L2, L3, and L4. The gill filaments were separated from the gill bar by cutting as closely as possible to the gill bar to allow the filaments to float freely. Before discarding, each gill bar was examined for larvae by manipulation with dissecting needles under the low and medium powers of a binocular dissecting microscope, and the larvae counted. Filaments containing larvae and any larvae that became detached from the filaments were collected in 5% formalin in separate vials for each arch. The formalin in the jar that originally contained the set of gills was also carefully checked in small quantities in a flat translucent dish under the microscope, and any loose larvae were counted and collected in a vial as "loose" larvae.

During the course of counting the larvae on the gill filaments, some lumpfish gills were examined for the relative numbers of larvae less than and greater than 5 mm from the tips of the filaments. This distance was determined by the use of a linear eyepiece micrometer scale that was calibrated against a stage micrometer.

Further investigation involved the determination of the developmental stages of the collected larvae using mainly Sproston (1942) for reference. Using the high power of a binocular dissecting microscope, nearly all the larvae could be assigned to one of the developmental stages 2–7 and the sex of stage-7 individuals could be readily determined. Developmental stage 2 is a copepodid, stages 3–6 are attached chalimus stages, and the stage-7 male is the final mature adult. The stage-7 female is similarly well developed, potentially free swimming, and ready for copulation but not yet sexually mature and metamorphosed to final adult form. Sproston (1942) produces evidence that the stage-7 female undergoes another molt into stage 8 on transfer to its final host and before metamorphosing, and if so, it can still be considered to be a larval form in stage 7. It will usually be convenient in this paper to refer to all stages on the intermediate host including (nominally) stage-7, as larval stages, to save space and to distinguish them from adult *L. branchialis* on the final host.

For sets of lumpfish gills containing up to about 200 larvae, and occasionally for larger numbers, all larvae in suitable condition were staged. For specimens containing larger numbers of larvae, the procedure was to select for staging one or more vials from among the 8 vials of larvae normally available for each set of lumpfish gills; the numbers of larvae examined in this way usually ranged from 100 to 200 for each lumpfish specimen, but as many as 1100–1200 larvae were sometimes examined. The method of selecting one or more vials, rather than proportional numbers from each vial of a set, was justified, because the data from specimens, for which all larvae were staged, showed no significant difference in the proportions of the various larval stages on the different branchial arches. Even when the initial examination of a set of gills yielded a significant number of "loose" larvae, the checking of a few of the vials containing "loose" larvae indicated that the proportional distribution of larval stages was not significantly different from that on the associated branchial arches.

Adult *Lernaeocera branchialis* on Cod

During 1947–70 over 80,400 of the Atlantic cod, *G. morhua*, sampled by St. John's Biological Station scientists and technicians, were checked for the presence of metamorphosed adult *L. branchialis*. This was done by turning the cod on its back and spreading the opercula and gills widely so that the copepod could be observed, if present, in its usual position at the ventral anterior apex of the branchial arches. This examination was not detailed enough to detect the "pennella" stage, but, on the few occasions when these were found in special examinations, data on their occurrence were not included with the data on the occurrence of the adult copepod. Also excluded from the results of this study was evidence of dead and decaying copepods and stalks of dead copepods from earlier infections.

Cod were examined for the presence of adult *L. branchialis* at West Greenland and over the Newfoundland, Labrador, and northern Gulf of St. Lawrence inshore and offshore areas; occasional cod samples from the southern Gulf of St. Lawrence and from the Scotian Shelf to Georges Bank were also examined for the presence of the parasite (Fig. 3–7; Table 6).

Most of the cod samples were random from research vessel catches or from commercial fishing but, in recent years, some samples collected by length categories were included.

Fish lengths in this paper are from the snout to mid-fork of the caudal fin for cod and total length for lumpfish, measured to the nearest centimeter.

Lernaeocera branchialis Larvae on Lumpfish Gills

Seasonal Distribution and Abundance

In the inshore areas (Fig. 2; Table 1), nearly all the lumpfish examined for *L. branchialis* larvae during April to August were infected (but 55% in May at Bonavista). The percentage infection was low (0–23%) in the south coast bays during September–December and ranged from 0 to 50% in the offshore Newfoundland areas. The two lumpfish taken on Dana Bank off West Greenland in August were both infected.

TABLE 1. *Lernaeocera branchialis* larvae on the gills of lumpfish (*Cyclopterus lumpus*) mainly in the Newfoundland area. (Numbers of lumpfish whose gills were examined for larvae in parentheses.)

	Inshore Newfoundland areas					
Months	Bonavista (A)	St. John's (B)	West coast and Strait of Belle Isle (C)	South coast (D)	Offshore Newfoundland areas (E)	Dana Bank off West Greenland (F)
	% lumpfish hosts infected with L. branchialis larvae					
Jan.	—	—	—	—	14(63)	—
Feb.	—	—	—	0(3)	20(15)	—
Mar.	—	—	—	—	13(16)	—
Apr.	—	—	—	100(2)	4(24)	—
May	55(11)	100(1)	100(7)	—	9(22)	—
June	94(17)	100(29)	100(1)	—	47(17)	—
July	100(9)	100(15)	100(6)	—	0(1)	—
Aug.	100(6)	100(10)	—	—	—	100(2)
Sept.	—	—	—	14(7)	50(4)	—
Oct.	100(1)	—	—	6(16)	40(15)	—
Nov.	0(1)	—	—	23(13)	—	—
Dec.	—	—	—	0(1)	0(1)	—
	Avg. no. L. branchialis larvae per lumpfish examined					
Jan.	—	—	—	—	0.1(63)	—
Feb.	—	—	—	0.0(3)	0.5(15)	—
Mar.	—	—	—	—	0.1(16)	—
Apr.	—	—	—	14(2)	0.1(24)	—
May	2(11)	5(1)	24(7)[a]	—	0.2(22)	—
June	120(17)	184(29)	131(1)[b]	—	10(17)	—
July	125(9)	1700(15)	636(5)[c] 4(1)[d]	—	0.0(1)	—
Aug.	594(6)	5697(3)[e] 90(7)[f]	—	—	—	158(2)
Sept.	—	—	—	0.1(7)	2(4)	—
Oct.	16(1)[g]	—	—	0.1(16)	0.7(15)	—
Nov.	0(1)[h]	—	—	0.2(13)	—	—
Dec.	—	—	—	0.0(1)	0.0(1)	—
	Range of numbers L. branchialis larvae infecting lumpfish gills					
Jan.	—	—	—	—	0–1	—
Feb.	—	—	—	0	0–6	—
Mar.	—	—	—	—	0–1	—
Apr.	—	—	—	3–25	0–2	—
May	0–4	5	1–42[a]	—	0–2	—
June	0–1550	1–807	131[b]	—	0–117	—
July	3–519	11–6113	127–1177[c] 4[d]	—	0	—
Aug.	1–2729	441–14040[e] 30–213[f]	—	—	—	1–314
Sept.	—	—	—	0–1	0–5	—
Oct.	16[g]	—	—	0–1	0–3	—
Nov.	0[h]	—	—	0–1	—	—
Dec.	—	—	—	0	0	—

[a]Estuary Little Codroy River; [b]Red Bay, Labrador; [c]Flowers Cove; [d]Henley Harbour, Labrador; [e]St. John's, August 2–7; [f]Bay Bulls, August 18; [g]Indian Bay; [h]Exploits Arm. For south coast areas see Fig. 1 and 2.

In inshore areas (Table 1, A, B, C; Fig. 2), the average number of *L. branchialis* larvae per lumpfish was low in May at Bonavista, St. John's, and on the west coast of Newfoundland but increased rapidly during the summer. There were large numbers of larvae on lumpfish at St. John's on August 2–7, but only small numbers at Bay Bulls, near St. John's, on August 18. No lumpfish

TABLE 2. Percentage monthly composition of *L. branchialis* larvae by stages on the gills of lumpfish in various parts of the Newfoundland area (A–E) and off West Greenland (F), 1951–70.

	Area	Month	Percentage composition of *L. branchialis* by larval stages 2	3	4	5	6	7	Total no. of larvae on gills (% *staged*)	No. of hosts from which larvae staged
A.	Bonavista	May	100	0	0	0	0	0	19(89)	6
	(inshore)	June	92	5	1	1	1	0	2,039(34)	16
	"	July	29	32	19	12	8	2	1,125(97)	9
	"	Aug.	11	4	8	6	27	44	836(100)	5
	"	Oct.	81	0	6	0	0	13	16(100)	1
B.	St. John's	May	80	0	0	0	0	20	5(100)	1
	(inshore)	June	61	22	9	7	2	1	4,983(40)	28
	"	July	35	14	10	13	16	13	19,118(17)	11
	"	Aug.	6	2	3	3	21	65	17,722(14)	10
C.	West coast	May	37	15	9	18	9	11	171(100)	7
	(inshore)	June	100	0	0	0	0	0	131(100)	1
	"	July	2	4	4	4	6	80	3,186(93)	6
D.	South coast	Apr.	96	0	0	0	0	4	28(100)	2
	(inshore)	Sept.	0	0	0	0	100	0	1(100)	1
	"	Oct.	0	0	0	0	0	100	1(100)	1
	"	Nov.	67	0	0	0	0	33	3(100)	3
E.	Offshore areas	Jan.	44	0	22	0	11	22	9(100)	9
	(Labrador,	Feb.	38	0	13	13	13	25	8(100)	3
	east Nfld.,	Mar.	0	0	0	0	50	50	2(100)	2
	Grand Bank,	Apr.	0	50	0	0	50	0	2(100)	1
	south coast	May	67	0	0	0	33	0	3(100)	2
	Nfld. banks,	June	6	16	1	3	32	41	154(100)	7
	and Gulf of	Sept.	17	0	0	0	33	50	6(100)	2
	St. Lawrence)	Oct.	45	0	0	0	18	36	11(100)	6
F.	West Greenland (Dana Bank)	Aug.	0	0	2	2	15	81	315(100)	2

were available after July for the west coast and after August at St. John's. No lumpfish from Bonavista were examined after August. Two lumpfish were examined from bays north of Bonavista (and included under Bonavista, Table 1); one of these had a low infection in October and the other taken in November was not infected. The level of infection was much higher at St. John's than at Bonavista in July–August and than on the west coast in July. In view of the small numbers of lumpfish examined, the wide variation in the numbers of larvae on lumpfish from the same area, and the very great number of larvae on a lumpfish from Bonavista in June, described below, the differences may only be due to chance. Inclusion of this specimen would increase the Bonavista average number of larvae per lumpfish for June (Table 1) to 1594, almost 3 times the Bonavista average for August and almost as high as the St. John's average for July.

Lumpfish taken in the bays along the south coast (Table 1, D) in spring and autumn showed a very low infection with *L. branchialis* larvae, the highest level occurring in April for 2 specimens taken near the shore in less than 15 m of water. Most of the south coast specimens were taken by otter trawl in 180–255 m during the autumn when the lumpfish, after spawning, had migrated from the shallow water near the shore to the deeper areas of the bays.

Only very small numbers of larvae were found in any month on the gills of lumpfish from the Newfoundland offshore areas (Fig. 2; Table 1, E). Larvae found on lumpfish gills in winter and early spring were well pigmented and apparently as normal as those taken in summer.

Very large numbers of larval *L. branchialis* occurred on the gills of some lumpfish. The greatest numbers (in Table 1) were 5563, 6113, and 14,040 larvae counted on 3 lumpfish taken at St. John's in July and August. More than 26,700 larvae were estimated on the gills of a lumpfish taken in 5 m near the shore at Bonavista on June 20, 1951, but these are not included in the data of Table 1 as the estimate was based on the number of larvae counted on the gills of only two branchial arches of a poorly preserved set of gills.

Kabata (1958) reported the largest number of larvae of *L. obtusa* from the gills of a lemon

sole, *M. kitt*, from the North Sea to be 577. The number of larvae of *L. branchialis* on lumpfish, *C. lumpus*, of the Murman area was as high as 362 per fish (Polyanskii 1955). Boxshall (1974) found the flounder, *P. flesus*, in the North Sea to carry as many as 550 larvae of *L. branchialis*.

In the Newfoundland east coast areas (Bonavista and St. John's), all but one of the few *L. branchialis* larvae found in May were in the second stage, the copepodid stage at which they first become attached to the lamellae of the gills (Table 2, A, B). Judging from the prevalence of late stage larvae on the gills of lumpfish taken offshore in January to April (Table 2, E), the single stage-7 specimen found at St. John's in May may have attached as a stage-2 larva in the previous year to a host lumpfish which lived in cold water in winter and spring. In the areas of high infection (A and B), development through the various larval stages appeared to be rapid, with 44% at Bonavista and 65% at St. John's having reached stage 7 by early August. Progression from stage 2 in May to stages 6 and 7 in August was apparently more rapid in the St. John's area than in the more northerly Bonavista area. In the warmer water off the west coast, the production of later stages and presumably the attachment of stage-2 larvae may have been as much as a month earlier than at St. John's (Table 2, C). Two lumpfish (of Table 2, C), taken on June 24 and July 17 close to the shore at Red Bay and Henley Harbour, Labrador, carried 131 and 4 *L. branchialis* larvae, all in stage 2. Development of these larvae was much less advanced than in the warmer coastal water off the west coast of Newfoundland in May and July (the remaining data of Table 2, C). On Dana Bank off West Greenland (Table 2, F), 81% of the larvae on 2 lumpfish were in stage 7 on August 19.

In the offshore Newfoundland areas, the prevalence of some larvae in the early and later stages of development in January–March indicates that they were probably carried over the winter from the previous year. However, the occurrence of stage-2 larvae on the gills of lumpfish taken during the autumn in several areas suggests that some free-floating stage-1 (nauplius) larvae are prevalent during most of the year in areas where the adult parasite is present. In the areas of high infection, the numbers in stage 7 probably reach a peak during the latter part of the summer.

Relative Numbers of Stage-7 Males and Females on Lumpfish Gills

The numbers of stage-7 *L. branchialis* on lumpfish gills from the Newfoundland area (Table 2, A–E), and the percentages of females at this stage, were as follows:

Months	No. in stage 7	% female
Jan.–Apr.	6	33
May	20	30
June	93	44
July	5059	42
Aug.	11847	36
Sept.–Nov.	11	82
Total	17036	38

Thus, apart from the small number of stage-7 individuals in the September–November period, females were consistently fewer than males. This is similar to Kabata's (1958) experience with *L. obtusa* on the gills of the lemon sole, where of 227 individuals in stage 7, 30% were female.

Sproston (1942) showed that males of *L. branchialis* from the gills of the flounder, *P. flesus*, were shorter than females at stages 5 and 6 and distinctly shorter at stage 7. It is therefore likely that the male passes through the earlier stages more rapidly than the female. As Kabata (1958) suggests for *L. obtusa*, it may be that the duration of stage 7 on the gills of the intermediate host is greater for the male than for the female, thus accounting in part for the relatively larger percentage of males in this stage. Although stage-7 males are occasionally found on the final host together with the young female (Kabata 1958, for *L. obtusa*), the females upon being fertilized become detached from the gill filaments of the intermediate host to seek a final host, whereas the male may remain on the gills of the intermediate host and fertilize several females before completing its life cycle. Scott (1901) says that female *L. branchialis* in the final (seventh) stage on the intermediate host, are much more pelagic than males and that the males remain on the flounder after the females have gone. In the wastewater of the Piel hatchery (on the NW coast of England), the ratio of stage-7 females to males was 25 to 1.

On the gills of 10 lumpfish (1 in June and 9 in July) bearing copulatory groups of stage-7 *L. branchialis*, there were 529 pairs (1♂, 1♀; attached in a manner similar to those of Capart 1948, fig. 14; Kabata 1958, fig. 4, in coitu; and Ho 1966, fig. 76, in amplexus), 36 triplets (2♂, 1♀), and 1 quadruplet (3♂, 1♀). Also, in two instances a male was attached to a female and another male attached to the first male.

Scott (1901) reported fertilization of stage-7 females of *L. branchialis* before the female had severed its chitinous connection with the gill filament of the intermediate host. Sproston (1942) described in detail copulations and transference

TABLE 3. Attachment distance of larval *L. branchialis* on lumpfish gill filaments. (M = male, F = female.)

Range	No. of lumpfish	Sex of lumpfish	Total larvae	Percent larvae <5 mm from tip of filament
30–34[a]	8	M	1850	58.2
35–39[a]	10	F	1227	61.9
40–44[a]	13	F	1214	63.8
45–49[a]	22	F	3705	57.5
50–54[a]	5	F	959	54.6
1–50[b]	35	—	422	54.5
51–200[b]	7	—	593	58.9
201–500[b]	9	—	2380	54.2
501–1000[b]	5	—	2890	59.7
1001–1550[b]	2	—	2670	62.7
Total[c]	58	M+F	8955	58.8

[a]Length range (centimeters) of lumpfish host.
[b]Range of numbers of larvae on one lumpfish.
[c]Totals for both a and b.

of spermatophores between male and female stage-7 *L. branchialis* but she did not describe the in amplexus pairing. Ho (1966) says that Sproston did not see paired examples and that her description of copulation is therefore a conjecture. Kabata (1958) found 26 copulatory pairs of male and female stage-7 *L. obtusa* (in coitu) on the gills of the lemon sole and concluded that fertilization of the female occurs while it is attached to the gill filament of the intermediate host. Ho (1966) presented convincing evidence that the immature female adult of *Cardiodectes*, a close relative of *Lernaeocera*, has a pair of spermatophores attached to its genital segment before it leaves the intermediate host.

LOCATION OF *L. branchialis* LARVAE ON THE GILL FILAMENTS

Nearly all of the *L. branchialis* larvae were situated along the distal half and most near the tips of the gill filaments. Of 8955 larvae from 58 lumpfish hosts ranging from 30 to 54 cm in total length, 59% were less than 5 mm from the tips of the filaments (Table 3). There was a slight decline in percentage of larvae less than 5 mm from the tips of the filaments for female lumpfish over the 40–44 to 50–54-cm length groups, probably due to the increasing length of the gill filaments; and there was a slight increase in the percentage for the groups containing the larger numbers of larvae. However, the variation in percentages was very small for both the length groups of the host (54.6–63.8%) and the ranges of larval numbers per lumpfish (54.2–62.7%). Kabata (1958) says that the great majority of larvae of *L. obtusa* are attached at or near the distal ends of the gill filaments of the lemon sole and that this suggests that the larvae move against the respiratory current.

NUMBER OF FILAMENTS ON THE BRANCHIAL ARCHES OF LUMPFISH

The filaments of branchial arches 1–4 were counted for eight mature lumpfish. The number of filaments per fish ranged from 1032 and 1204 and averaged 1118, the percentages on the left and right sides being 49.7 and 50.3, respectively. The percentages of filaments on branchial arches 1, 2, 3, and 4 were 28.3, 28.6, 29.3, and 13.9, respectively. The differences between the numbers of filaments on branchial arches 1, 2, and 3 were not significant, but these were all significantly greater ($P < 0.001$) than the number of filaments on the fourth arch. The fourth branchial arch has only a single lamella or hemibranch, whereas there are two hemibranchs making up the gill of each of branchial arches 1–3.

DISTRIBUTION OF *L. branchialis* LARVAE ON GILLS OF BRANCHIAL ARCHES 1–4 OF LUMPFISH

In the following comparisons, by χ^2 tests, of the actual numbers of larvae on the left and right gills of branchial arches 1–4, the larval counts were grouped according to the larval groups in the first column of Table 4. These groups are labeled P, Q, R, S, T, and U for simplicity in re-

TABLE 4. Distribution of *L. branchialis* larvae on gills of branchial arches 1–4 of lumpfish. (Specimens with more than 20% of the total larvae detached from the gill filaments are not included. L = left, R = right.)

Larval groups[a]		No. of lumpfish hosts	Total no. of larvae on gills and loose	% loose	Total no. of larvae on gills (L + R)	% of total larvae on left and right gills		% of total larvae on left and right gills of branchial arches 1–4			
						L	R	1	2	3	4
P	1–50	86	881	4.9	838	46.8	53.2	33.2	32.7	24.6	9.5
Q	51–100	9	686	5.0	652	49.5	50.5	37.2	31.4	20.9	10.4
R	101–200	10	1,330	5.5	1,257	45.3	54.7	33.4	32.2	26.0	8.4
S	201–400	10	2,835	7.0	2,637	47.9	52.1	36.3	33.9	23.3	6.5
T	401–807	10	6,258	10.3	5,615	49.6	50.4	34.7	34.7	22.8	7.8
U	1177–2609	4	6,645	6.0	6,245	45.9	54.1	37.3	31.5	24.5	6.6
V	5563	1	5,563	7.3	5,155	47.3	52.7	32.3	35.8	23.9	7.9
W	14040	1	14,040	9.7	12,678	46.3	53.7	16.5	42.7	30.3	10.5
P–T	1–807	125	11,990	8.3	10,999	48.5	51.5	35.0	33.9	23.3	7.8
P–U	1–2609	129	18,635	7.5	17,244	47.5	52.5	35.8	33.0	23.8	7.4
P–W	1–14040	131	38,238	8.3	35,077	47.0	53.0	28.3	36.9	26.2	8.6

[a]Allocation to a larval group was based on the total number of larvae on right and left gills plus any detached larvae found with the set of gills.

ferring to them in the statistical comparisons. The last two categories (V and W) represent individual specimens whose larval counts were not included in the grouped comparisons because they would tend to swamp any differences that might be apparent in groups P–U.

Relative numbers of larvae on left and right gills — The relative proportions of larvae on the gill filaments of the left branchial arches 1–4 for the individual groups P–U were compared with the corresponding data for the right branchial arches (Table 4). For groups R, S, T, and U, the distribution of larvae on the left branchial arches was not significantly different from that on the right ($P > 0.05$). For groups P and Q, the left and right distributions were barely significantly different ($P < 0.05 > 0.02$) when these were treated individually; but upon combining the two groups the left and right distributions were very similar ($P > 0.95$). The reason for this is that the proportional larval distribution on the left branchial arches of group P was almost identical with that on the right branchial arches of group Q, and vice versa. When the data for larval groups P–U were combined (2940, 2698, 1928, and 633 larvae on the left branchial arches 1–4, respectively, vs. 3238, 2997, 2168, and 642 larvae on the corresponding right branchial arches), there was no significant difference between left and right in the relative proportions on the gills of the 4 branchial arches ($P > 0.30$).

Although the proportional distribution of larvae on the left and right gills of branchial arches 1–4 was not considered to be significantly different, the number of larvae on the left side (branchial arches 1–4 combined) was consistently less than the number on the right side. Even when the numbers of larvae on left and right sides were compared for the 32 pairs of values represented by the 4 branchial arches and the 8 larval groups (of Table 4), in 24 cases the number of larvae on the left side was less than that on the right and in 3 cases the numbers were equal.

Comparing the relative numbers on the left and right sides for the larval groups P–U (Table 4), the difference was significant for group S ($P < 0.05$), for groups R and U ($P < 0.001$), and for P–U combined ($P < 0.001$). The two individual hosts (V and W) with very large numbers of larvae also had larger numbers of larvae on the right gills than on the left.

The reason for the greater number of larvae on the right than on the left side of the gills is not readily evident, as the numbers of gill filaments on the right and left branchial arches were not significantly different in the eight lumpfish examined, nor was there a noticeable difference in the length and surface area of left and right filaments. The fact that an average of about 7% of the larvae were detached from the gill filaments detracts somewhat from the clarity of the statistical comparisons, but there seems to be no reason why more larvae should become detached from the left than from the right gills during the process of preservation and examination of the gills.

Distribution of larvae on the gills of individual branchial arches 1–4 — Because the proportional distribution of larvae on branchial arches 1–4

was similar for both the right and left sides, the numbers of larvae on the right and left sides were combined for each of the 4 branchial arches (Table 4). In all groups of P–U except T where the numbers were equal, larvae were slightly more numerous on gills of the first than of the second branchial arch, but the difference was significant only for group U and for the total of P–U. For all groups and for the total, the numbers on arch 2 were significantly greater than those on arch 3, and the numbers on arch 3 significantly greater than on arch 4 ($P < 0.001$ in each case). For the largest two larval numbers (Table 4, V, W) not included in the above groups, the numbers on the first arch were less than on the second and for the largest number greatly less.

Treating the groups individually in comparing the proportional distribution of larvae on the gills of branchial arches 1–4, the following results were obtained by χ^2 tests (using actual numbers of larvae on branchial arches 1–4):

Groups compared	*P*
P vs. Q	>0.20
PQ vs. R	>0.10
PQR vs. S	<0.01
PQR vs. T	<0.05
PQR vs. U	<0.001
PQR vs. STU	<0.001

The distribution of larvae on branchial arches 1–4 for larval groups P, Q, and R combined differed very significantly from the distribution for groups S, T, and U combined ($P < 0.001$). The relative numbers for groups P, Q, and R (34.3, 32.2, 24.3, 9.2%) declined from gill arches 1–4 at a slower rate than for groups S, T, and U (36.1, 33.2, 23.6, 7.1%). It seems possible that, when large numbers of larvae are present, the effect of crowding may reduce the possibility of attachment on the fourth gill arch which contains the shortest and the least number of filaments. However, the specimen with the largest number of larvae (Table 4, W) had 10.5% of the larvae on the gills of the fourth branchial arches, but this fish had only about half the usual percentage on the first branchial arches, thus increasing considerably the percentages for the other arches.

The small number of gill filaments on the fourth branchial arch, only about half of the number on branchial arches 1–3 and with slightly shorter filaments and consequently lesser surface area, offers a partial explanation for the much smaller numbers of larvae on the gills of the fourth branchial arch. The absence of a fifth gill slit in the lumpfish and the consequent lack of a water current posterior to the fourth branchial arch must also contribute to the reduced abundance of larvae on the fourth arch. The somewhat smaller number of larvae on the gills of the third branchial arch than on those of arches 1 and 2 was apparently not due to fewer filaments on the third arch. From observations on four lumpfish (31–51 cm long), the surface area of the gill filaments on the third arch was estimated to be slightly smaller than that of the second arch and the fourth gill slit to be slightly smaller than the other three. However, these differences do not appear to be enough to account fully for the smaller numbers of larvae on the gills of the third branchial arch. The gills of the first branchial arch have some advantage in the reception of larvae. The gill area appears to be slightly larger than that of the second arch and the most anterior hemibranch has the sole advantage of receiving larvae which pass into the area of the first gill slit, whereas the openings of gill slits 2, 3, and 4 are each faced by two hemibranchs.

Adult *Lernaeocera branchialis*

Infection of Cod by Adult *L. branchialis* at Different Times During the Year

Infection rates are available throughout the year for only a few areas (Table 5). In the various areas and length ranges, the highest percentage infection ranges throughout all seasons of the year and depends considerably on the relative numbers of sampled cod which at an earlier period were related to the coast.

Suitability of *L. branchialis* as an Indicator Tag for Cod Migrations

The effectiveness of *L. branchialis* as an indicator tag for cod movements depends on the suitability of the life history of the parasite for this purpose. The area of infection of the final host, the cod, should be limited. The available evidence indicates that abundant stage-7 females of *L. branchialis* on the intermediate host occur mainly in July–August in inshore waters of the east coast of Newfoundland. Abundant seventh-stage females very likely occur up to a month earlier in inshore areas on the west coast of Newfoundland and at least several weeks later in inshore Labrador waters than on the east coast of Newfoundland. Seventh-stage females on lumpfish gills were plentiful near the coast, but in areas distant from the coast lumpfish carried very few larvae. Moreover, the stage-7 female, already fertilized when it becomes free from the intermediate host, apparently is planktonic for only a short

TABLE 5. Percentages of Atlantic cod (*Gadus morhua*) of the Newfoundland area, 1947–70, infected with adult *L. branchialis* at different times during the year. (Numbers of cod examined in parentheses.)

Monthly period	Percentages of cod infected with *L. branchialis* at centimeter length ranges below (no. cod examined)		
	31–50	51–70	71–90
	Division 3L inshore and coastal shelf		
Jan.–Apr.	5.0 (120)	2.8 (528)	1.8 (438)
May–July	10.4 (1617)	6.2 (3649)	3.3 (3043)
Aug.–Sept.	5.4 (463)	3.4 (1080)	1.6 (499)
Oct.–Dec.	9.9 (776)	4.1 (1379)	2.5 (1142)
	Division 3Ps N of 46° N		
Jan.–Apr.	13.6 (176)	9.5 (693)	3.7 (322)
May–July	9.3 (724)	8.1 (937)	4.1 (393)
Sept.–Dec.	16.4 (287)	7.8 (307)	6.3 (80)
	Division 4R		
Jan.	2.7 (224)	7.2 (305)	—
June–July	10.4 (1447)	5.4 (1249)	1.7 (344)
Aug.–Sept.	10.5 (143)	4.3 (398)	3.4 (207)
Oct.–Dec.	6.1 (408)	5.7 (383)	6.4 (78)

period, at the most a few days (Stekhoven and Punt 1937; Sproston and Hartley 1941; Capart 1948; Kabata 1958). Thus it has a short range and in the Newfoundland and Labrador area the coastal currents have an onshore set. These females liberated near the coast should, therefore, infect cod near the coast. These cod should usually be small because the smaller cod throughout the summer and early autumn keep closer to shore than the larger cod; but in late June–early July when capelin are spawning, cod of commercial size often approach the beaches chasing capelin (Templeman 1948).

The adult phase of the *L. branchialis* parasite on cod apparently also lasts long enough to provide information on the yearly migrations of this fish. Our data (Table 5) show high year-round infection rates of cod with the adult copepods. Also, with by far the major infection of cod apparently occurring inshore in summer and with cod absent from the shallow inshore water in winter, the adult parasite must have a minimal life cycle of about a year to provide larvae to settle on the lumpfish gills in the shallow inshore water during the following summer. This copepod is capable of producing more than one batch of eggs (Heegaard 1947); the life of the closely related *L. obtusa* on haddock in the North Sea was estimated at 1½ yr and its adult reproductive phase about a year (Kabata 1958).

The lumpfish, which spawns in inshore waters in summer, and whose gills become infected with *L. branchialis* at this time, is the only intermediate host of the copepod yet discovered in the western Atlantic. Judging from European experience (Sproston and Hartley 1941; Capart 1948), and the distribution of infection by the adult copepod, if any other intermediate host of this copepod exists in this area it is likely to be a fish that lives near the coast in late May, summer, and early autumn.

INFECTION OF COD OF VARIOUS LENGTH RANGES WITH ADULT *L. branchialis* ESPECIALLY AS INDICATIVE OF COD MIGRATIONS

Infection rates of cod with *L. branchialis*, in areas associated with the coast, usually increased from the smallest to intermediate lengths and declined rapidly at greater lengths (Table 6). In many areas, cod above 80 cm were not infected, and in most areas not above 90 cm. Cod greater than 110 cm in length were not infected.

The offshore Labrador and northeast Newfoundland (ICNAF Divisions 2J, 3K) cod populations had considerably lower infection rates at the smaller sizes than the inshore populations of these divisions but had approximate equality at the lower infection rates of the larger sizes (Table 6, Fig. 3–7). Granted that the infection usually occurs near the coast, these infection rates indicate that the larger offshore and inshore fish were from the same intermingling population but that the smaller offshore fish did not visit the coast in sufficient numbers to obtain high infection rates. This observation helps to explain the relative

TABLE 6. Numbers of cod in various ICNAF divisions, 1947–70, examined for adult *L. branchialis* and percentages of these cod infected with one or more of these copepods.

Length range (*cm*)	1B, 1C, 1D (W. Greenland banks)[a]	2J, 2H, 2G (Labrador inshore)	2J, 2H, 2G (Labrador offshore)	3K (NE Nfld., inshore)	3K (NE Nfld., offshore)	3L (E and SE Nfld., inshore and coastal shelf)	3L (N Grand Bank, offshore W of 50°W)	3L (N Grand Bank, offshore E of 50°W)	3M (Flemish Cap)	3N, 3O (S Grand Bank)	3P (north of 46°N)	3Ps (St. Pierre Bank, south of 46°N)	4R (N Gulf of St. Lawrence)	4S (N Gulf of St. Lawrence)	4T (S Gulf of St. Lawrence), Scotian Shelf, Georges Bank	Total all areas except 3M
							Numbers examined									
11–20	0	554	70	21	29	44	70	11	32	487	89	100	112	2	0	1,589
21–30	12	199	583	74	254	138	201	70	37	1,048	232	251	255	25	49	3,391
31–40	52	568	1,640	131	591	860	231	142	126	1,325	429	277	981	107	166	7,500
41–50	284	2,723	2,136	217	1,093	2,196	374	325	262	1,463	758	291	1,241	296	407	13,804
51–60	430	6,641	2,260	621	996	2,656	424	395	282	1,560	1,055	247	1,333	612	477	19,707
61–70	302	4,171	1,189	673	463	4,043	529	544	102	1,117	882	167	1,002	403	215	15,700
71–80	234	1,665	261	300	225	3,360	539	312	46	987	519	105	481	211	55	9,254
81–90	230	847	47	94	114	1,760	262	119	19	669	356	50	239	58	26	4,871
91–100	69	442	22	27	40	920	112	36	5	344	127	25	109	21	10	2,304
101–110	4	133	10	6	10	388	82	13	5	163	40	11	53	10	3	926
111–160	0	43	6	5	6	124	60	3	14	107	27	10	33	9	2	435
							% infected									
11–20	—	1.1	1.4	14.3	0	2.3	10.0	0	0	0	2.2	2.0	8.9	0	—	2.0
21–30	0	10.1	2.1	2.7	3.1	5.8	6.0	1.4	0	0.1	1.3	1.6	12.5	4.0	8.2	3.2
31–40	5.8	8.5	1.6	9.2	1.2	8.3	10.0	0.7	0	0.7	10.7	0.7	8.3	0.9	3.6	4.5
41–50	8.5	4.7	2.1	11.1	2.3	9.4	4.5	2.2	0	1.2	12.1	2.7	9.3	5.1	1.2	5.3
51–60	3.5	1.3	1.8	2.7	2.6	6.4	4.0	2.3	0	0.5	8.7	3.6	5.9	3.9	0.8	3.0
61–70	3.6	0.9	1.5	1.6	0.9	4.0	3.0	0.6	0	0.5	8.4	3.6	4.6	0.7	0	2.5
71–80	0.4	1.1	3.1	1.0	0.4	3.2	1.7	0	0	0.2	4.8	1.9	3.3	1.4	0	2.1
81–90	0.4	0.8	0	0	0	2.0	0.4	0	0	0.3	2.2	8.0	0.8	1.7	0	1.3
91–100	0	0.2	0	0	0	0.3	0	0	0	0	2.4	0	0	0	0	0.3
101–110	0	0	0	0	0	0.3	0	0	0	0	2.5	0	0	0	0	0.2
111–160	—	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

[a]August 1965 including 13 cod from 1A.

failure of the cod fishery of coastal Labrador and northeastern Newfoundland when the numbers of larger, older cod of ICNAF Subarea 2 and Division 3K were greatly reduced by the offshore fishery. The inshore failure in the fishery occurred in spite of the presence in the related offshore areas of large populations of smaller, younger cod available to the winter–spring offshore fishery. For cod over 30 cm in length, infection rates near the coast were much higher off Newfoundland and off southern Labrador immediately north of the Strait of Belle Isle than farther north along the remainder of the Labrador coast.

In ICNAF Division 3L, cod from offshore west of 50°W (mainly northwest Grand Bank and slopes and Avalon Channel) had high rates of infection, similar to those of inshore cod, at the 21–40 cm range. At all higher length ranges they had lower rates than inshore cod, but higher at all length ranges, especially at 11–40 cm, than cod from east of 50°W. Granted again that most of the parasites were acquired by smaller fish living inshore, apparently the smaller of the offshore cod of Division 3L west of 50°W visited the shore as much as the cod of these sizes taken inshore in 3L. However, about half the offshore cod of the larger sizes from west of 50°W apparently migrated from areas where little or no direct infection occurred, such as ICNAF Division 3L (E of 50°W) and southern Grand Bank (ICNAF Divisions 3N and 3O).

The smaller and the larger northern Grand Bank cod, from Division 3L east of 50°W, had comparatively little infection by the copepod and consequently much less relationship with the coast than fish of intermediate length (41–60 cm) which had about half the infection of offshore fish west of 50°W and between one third and one quarter that of the coastal fish of 3L. The smaller fish are likely to be largely native to the area and many of the larger fish immigrants from the centers of low infection, Divisions 3N and 3O (Table 6, Fig. 3–7).

At Flemish Cap (ICNAF Division 3M), there was no infection at any length (Table 6, Fig. 3–7) although at the lengths 21–60 cm there were 76% as many cod examined at Flemish Cap as in the neighboring Division 3L, east of 50°W, which had 18 *L. branchialis*, an average of 1.9% infection at these lengths. This confirms indications

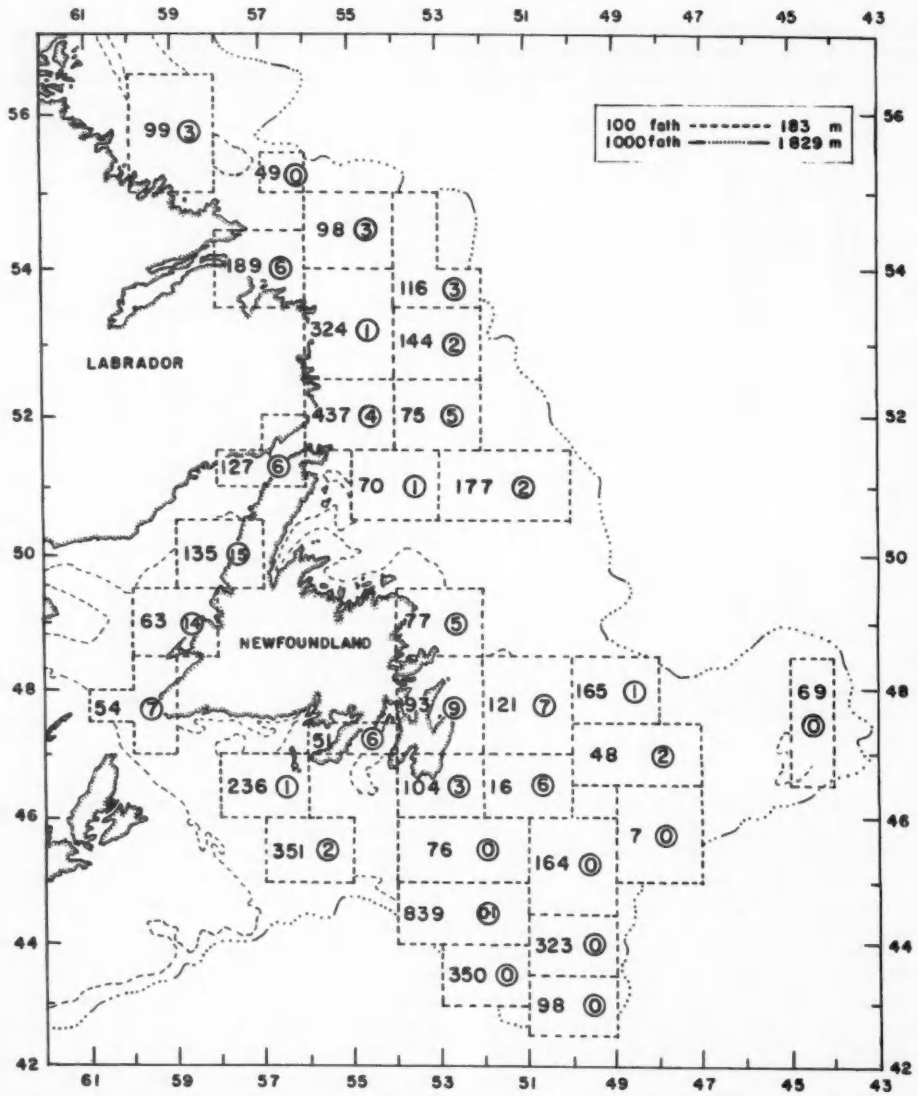

FIG. 3. *Lernaeocera branchialis*: distribution of infection of 11–30-cm Atlantic cod (*Gadus morhua*) by the adult parasite. (Free numbers = number of cod examined; Encircled numbers = percentages of these cod infected by one or more adult copepods.)

from earlier studies that Flemish Cap cod are a separate stock: lack of infection on Flemish Cap cod by *L. branchialis* (Templeman and Fleming 1963), and by the cod nematode, *Terranova (Porrocaecum) decipiens* (Templeman et al. 1957); considerably earlier cod spawning on Flemish Cap than on the adjacent Grand Bank (Templeman 1962); and cod tagging (Konstantinov 1970; Templeman 1974).

There was only a minor infection rate at all length ranges of cod on the southern Grand Bank, Divisions 3N and 3O (Table 6, Fig. 3–7). In the

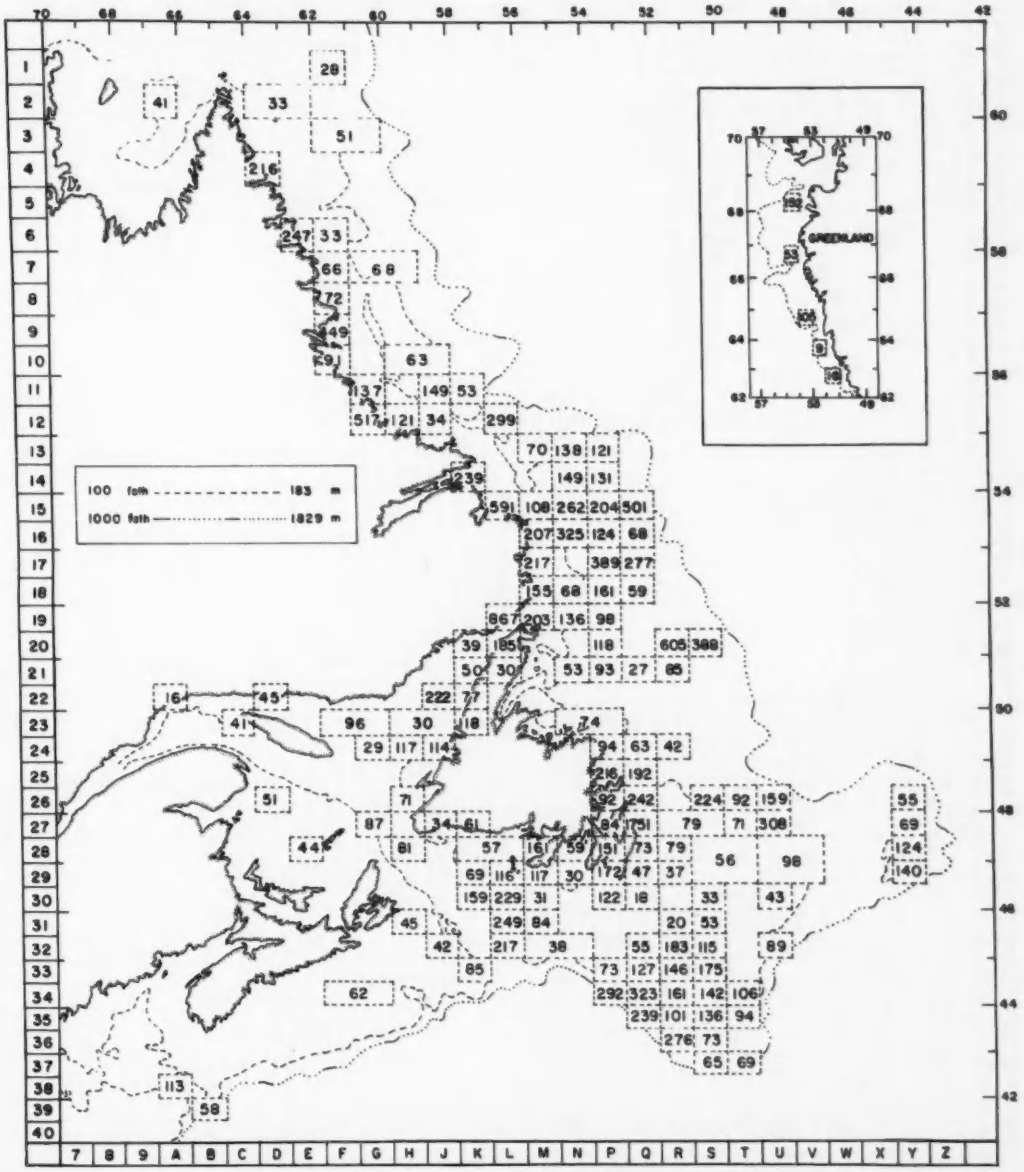

FIG. 4. *Lernaeocera branchialis*: distribution of infection of 31–50-cm cod by the adult parasite (numbers of cod examined).

11–30-cm group (Fig. 3), *L. branchialis* was not found in Division 3N and there was only one occurrence, <1% of the fish, in Division 3O. In the 31–50 and 50–90-cm groups (Fig. 4–5, 6–7), the rate of infection was noticeably higher in Divisions 3N and 3O and infection extended farther southward than in the 11–30-cm group. Infection for all length groups decreased in proportion to distance from the coast. In the 31–50-cm but especially in the 51–90-cm group (Fig. 4–7), infection rates were higher in Division 3O than in 3N which is more distant from the coast.

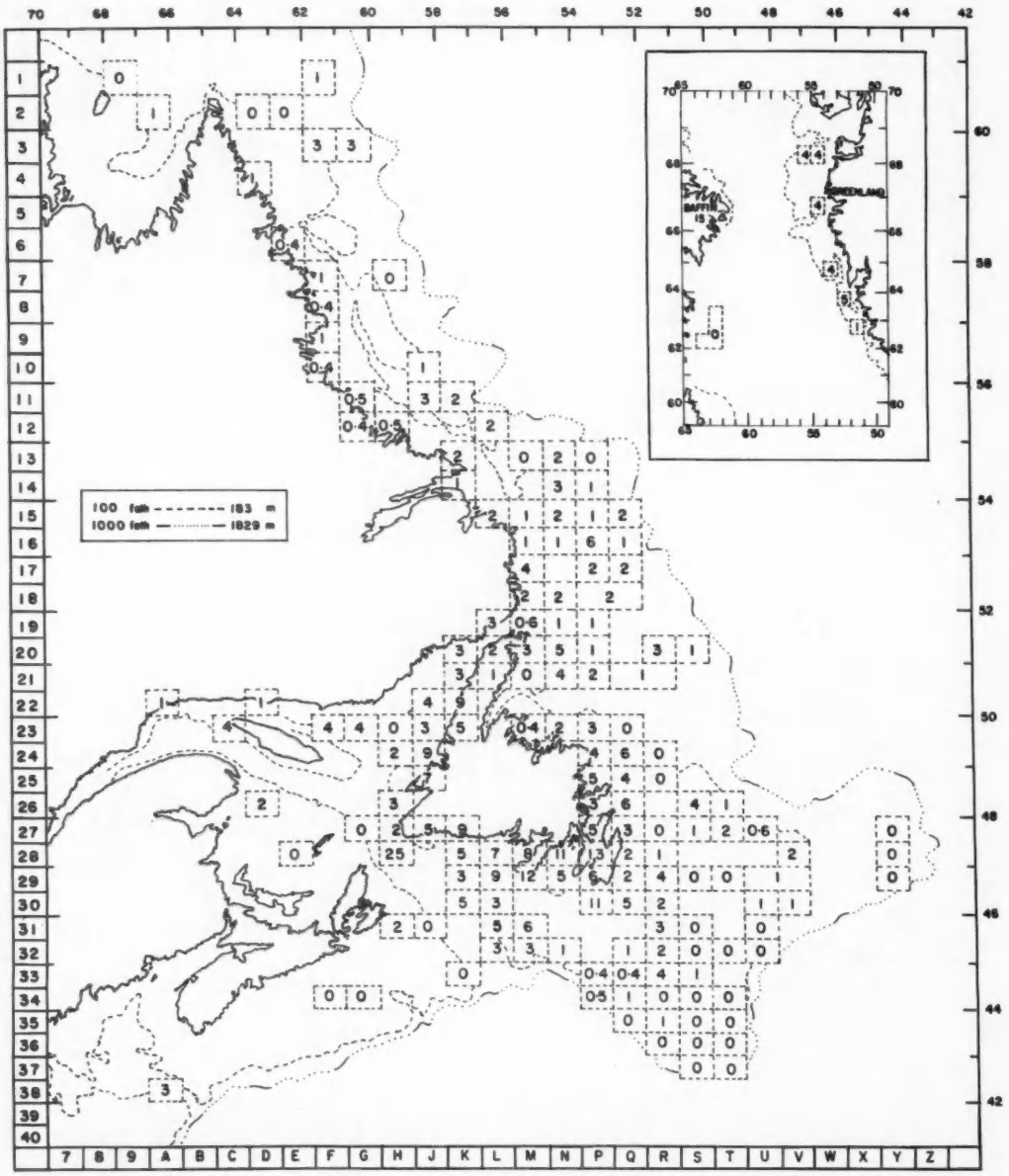

FIG. 5. *Lernaeocera branchialis*: distribution of infection of 31–50-cm cod by the adult parasite (percentages of cod infected by one or more parasites).

In these divisions the lowest infection rates were at the greatest distance from the coast.

Earlier studies of infection of cod with the cod nematode (Templeman et al. 1957), with *L. branchialis* (Templeman and Fleming 1963), and tagging of cod (Templeman and Fleming 1962; Templeman 1974) indicated only a minor association with the coast for cod of Divisions 3N and 3O. This association was greater from the western Division, 3O. Comparative ratios of in-

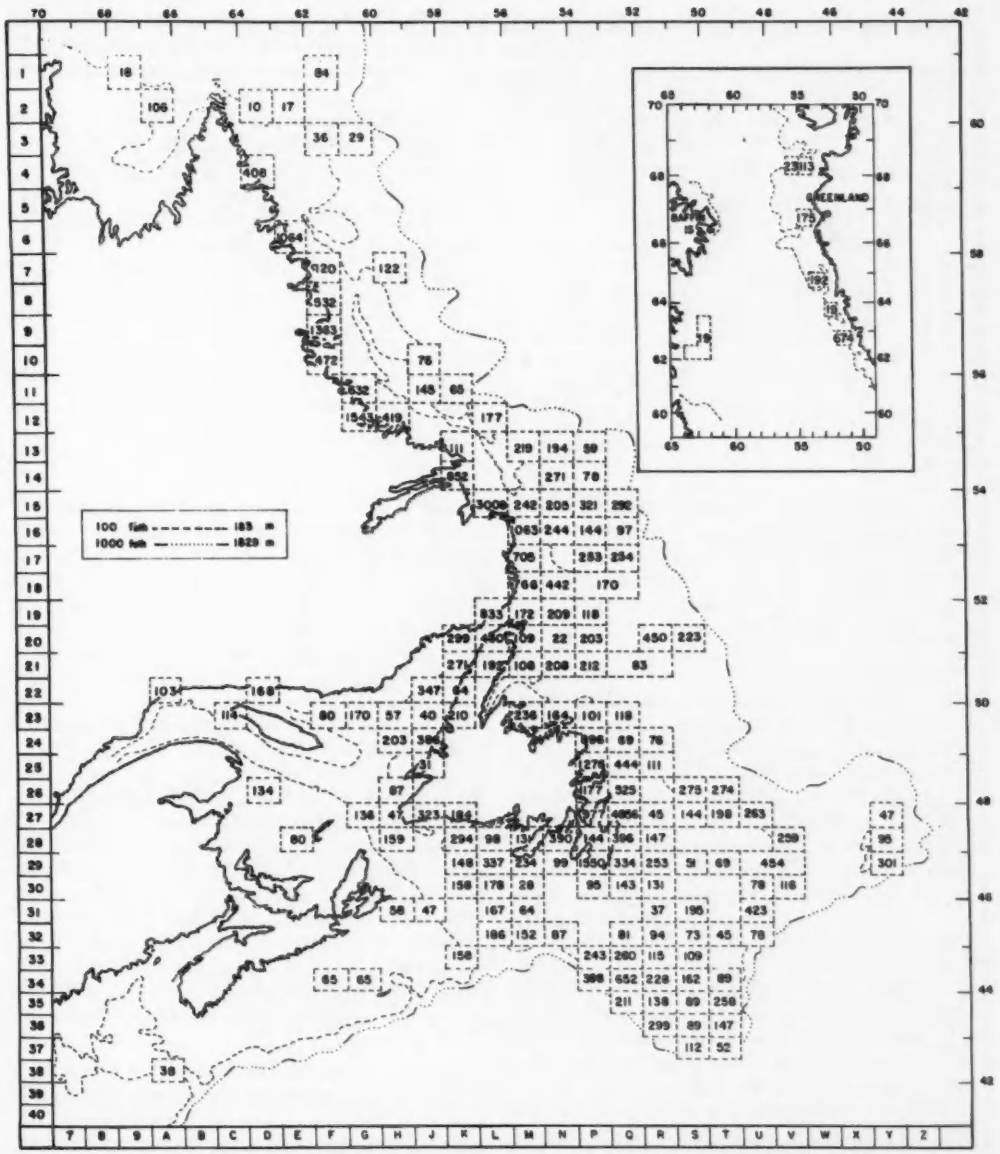

FIG. 6. *Lernaeocera branchialis*: distribution of infection of 51–90-cm cod by the adult parasite (numbers of cod examined).

fection (and therefore of association with shore) between cod of Divisions 3N and 3O and those of Division 3L inshore (Table 6) were quite similar (0.08–0.13 after combining 71–90 cm) at length ranges from 31 to 90 cm, but declined to 0.02 at 21–30 cm and to zero at 11–20 cm.

Cod of St. Pierre Bank south of 46°N (Table 6, Fig. 3–7) were much less infected with *L. branchialis* than those on the northern part of this bank, the neighboring banks of ICNAF Division 3P, and the south coast of Newfoundland. At the smallest lengths, 11–30 cm, infection was low and

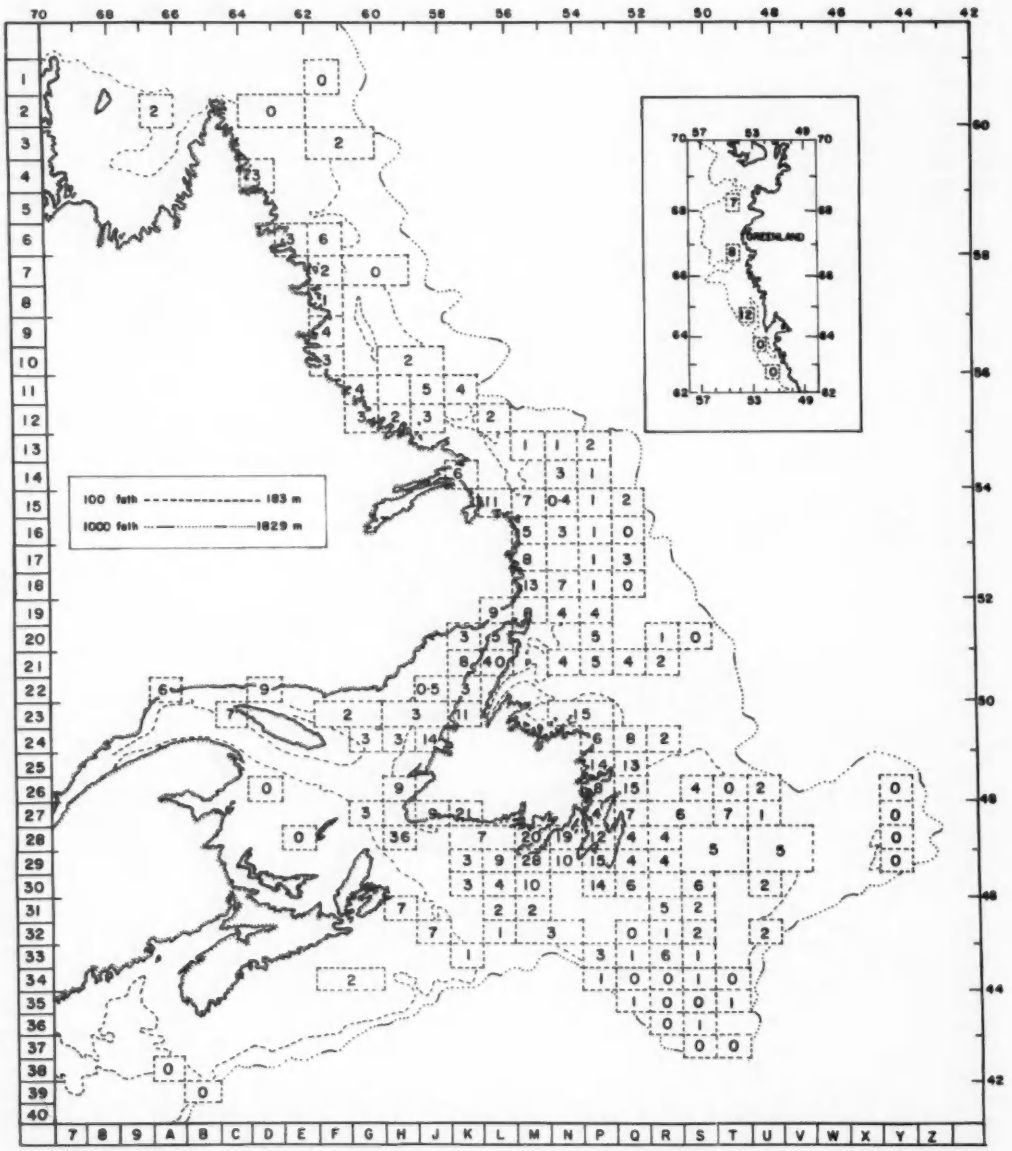

FIG. 7. *Lernaeocera branchialis*: distribution of infection of 51–90-cm cod by the adult parasite (percentages of cod infected by one or more parasites).

approximately the same in the southern and northern areas of St. Pierre Bank and higher near the adjacent coast. For cod 31–50 cm in length, unlikely to migrate in large numbers between the coast and the southern part of the bank, infection south of 46°N was comparatively low whereas close to the coast it was often very high. At still greater lengths there were increasing (although still lower) rates of infection in the southern compared with the northern part of the area,

TABLE 7. Rates of infection with adult *L. branchialis* for immature and mature cod of different length ranges in various ICNAF divisions, 1947–70. (Imm = immature, Mat = sexually mature.)

Length range (*cm*)	2G, 2H, 2J, 3K (Labrador and NE Nfld.)								3L (E and SE Nfld. inshore and coastal shelf, and offshore W of 50°W) 3P (N of 46°N)				4R (N Gulf of St. Lawrence)			
	Inshore				Offshore											
	Male		Female		Male		Female		Male		Female		Male		Female	
	Imm	Mat	Imm	Mat	Imm	Mat	Imm	Mat	Imm	Mat	Imm	Mat	Imm	Mat	Imm	Mat
Total no. cod examined for adult L. branchialis																
21–30	124	0	149	0	431	0	405	0	284	0	286	0	141	1	113	0
31–40	268	64	360	6	1101	63	1062	2	746	5	767	0	422	28	524	6
41–50	297	1308	880	443	508	1084	1398	230	1265	288	1686	24	346	278	515	99
51–60	56	3852	191	3154	38	1312	480	1408	541	1389	1453	671	101	545	261	410
61–70	3	1924	16	2899	1	518	34	1092	156	2441	462	2321	20	468	43	458
71–80	0	667	4	1289	2	153	1	326	31	2125	85	2139	0	240	8	232
% total cod infected with L. branchialis																
21–30	8.9	—	7.4	—	2.3	—	2.5	—	2.1	—	5.9	—	14.9	0	9.7	—
31–40	10.4	4.7	8.1	0	1.9	0	1.2	0	8.3	0	10.0	—	6.6	3.6	9.9	0
41–50	9.8	3.3	8.0	2.3	3.9	1.6	2.1	1.3	9.4	8.7	10.0	4.2	14.2	4.7	9.9	3.0
51–60	3.6	1.5	1.0	1.3	5.3	2.2	3.1	1.4	11.1	5.7	7.4	4.0	13.9	4.4	8.8	3.4
61–70	0	1.2	6.3	0.8	0	1.0	2.9	1.3	5.1	4.8	7.4	3.9	20.0	4.7	9.3	3.1
71–80	—	1.2	0	1.1	0	3.3	0	1.2	6.5	3.6	10.6	2.4	—	3.8	12.5	2.6

because larger cod are more migratory. Also, as shown by tagging results (Templeman 1974), large numbers of fish from coastal areas of the Avalon and Burin peninsulas, which have a relatively high percentage infected with the copepod, spend the winter–spring on St. Pierre Bank including the southern part of the bank. The very high infection rate of 8.0% for southern St. Pierre Bank at 81–90 cm (Table 6) was probably the result of the small sample size (50 fish).

The infection rate on the north shore of the Gulf of St. Lawrence (ICNAF Division 4S) was lower than that in the adjacent Division 4R off the west coast of Newfoundland. This was probably caused by the offshore location of more of the 4S samples whereas most of the 4R samples were coastal.

On the West Greenland banks, which are close to the coast, there were moderate rates of infection with *L. branchialis* (Table 6, Fig. 4–7). Infection rates in Ungava Bay were low, as off the adjacent northern Labrador coast.

COMPARATIVE RATES OF INFECTION OF IMMATURE AND MATURE COD OF VARIOUS LENGTHS WITH *L. branchialis*

For comparative rates of infection by *L. branchialis* of sexually immature and mature cod, all coastally related areas with high infection rates were considered and also offshore off Labrador and northeast Newfoundland where rates of infection were moderately low but fairly large numbers of fish were examined. Data from adjacent areas with approximately similar lengths at sexual maturity were combined.

Disregarding the data for less than 10 fish, the general picture (Table 7) is that sexually mature fish, both male and female, possessed considerably lower infection rates than immature fish of the same length range, and usually considerably lower than immature fish of the next higher 10-cm length range even when infection rates declined at the higher ranges. There are relatively higher numbers of mature than of immature fish at the upper than at the lower part of a 10-cm length range. Consequently, if there were no effect of infection on maturity, it would be expected that the infection rates of mature fish would be higher than those for immature at the same length range when infection is rising from one length range to the next, or to be approximately equal when the infection rates are similar from one length range to the next, but this does not occur.

Also, at length ranges where infection rates are declining with increase in length, it would be expected that mature fish of the same length range would have lower infection rates than the immature but they should not be lower than those of immature fish of the next higher length range.

The data, therefore, demonstrate that infection by *L. branchialis* interferes with the onset of sexual maturity and causes the fish to be immature at a larger size and presumably at a greater age, because infected fish should grow more slowly than those not infected.

There are other instances of parasites affecting sexual maturity of fishes: Kabata (1958) found that both male and female haddock of 24–33 cm (3-cm length groups) had smaller gonad weights in fish infected with *L. obtusa* than in uninfected fish. Hickling (1963) observed that mature female dogfish, *Etmopterus spinax*, parasitized by

TABLE 8. Percentages of infected cod from the northwest Atlantic 1947–70 carrying one or more adult *L. branchialis*.

Cod length range (*cm*)	Total cod infected with *L. branchialis*	Percentages total infected cod at each length range											
		1 *L. branchialis*				2 *L. branchialis*				3, 4, 5 *L. branchialis*			
		Male		Female		Male		Female		Male		Female	
		Imm	Mat	Imm	Mat	Imm	Mat	Imm	Mat	Imm	Mat	Imm	Mat
11–40	322	41.6	1.2	44.4	—	5.3	0	5.3	—	1.6	0	0.6	—
41–60	965	20.5	20.3	34.4	8.6	4.1	2.8	5.7	0.9	1.0	0.3	1.2	0
61–100	566	3.0	42.9	8.5	34.8	0.4	4.2	1.6	3.5	0.2	0.4	0.5	0
Total	1853	18.8	23.9	28.2	15.1	3.2	2.8	4.4	1.6	0.9	0.3	0.9	0

the cirripede, *Anelasma squalicola*, were never pregnant. Infection of the three-spined stickleback, *Gasterosteus aculeatus*, with the plerocercoids of the cestode, *Schistocephalus solidus*, delayed the sexual maturity of both sexes and prevented many from breeding (Pennycuick 1971).

NUMBER OF *L. branchialis* ON A FINAL HOST

Individual infected cod possessed 1–5 *L. branchialis* (Table 8). Percentages of the total of 1853 infected fish possessing 1, 2, 3, 4, and 5 of these copepods were 86.1, 11.9, 1.3, 0.6, and 0.05. Male cod, maturing at smaller sizes, had a smaller percentage of the total infected as immature and a larger percentage as mature fish than females. The ratios of mature fish to immature for *L. branchialis* numbers 1, 2, and 3–5 declined for males from 1.3 to 0.9 to 0.2 and for females from 0.54 to 0.36 to 0 (17 immature and no mature females with 3–5 copepods). The ratios of male cod to females with 1, 2, and 3–5 copepods per fish were 0.99, 1.00, and 1.24, the latter difference from equality being due to the absence of mature females with 3–5 copepods.

Mann (1952) noted the very high infection rate of 80% with *L. branchialis* in small cod of 10–ca. 30 cm originating from the vicinity of Cuxhaven to Helgoland in the southern part of the North Sea. The percentages with various numbers of *L. branchialis* were: 1 parasite (50%), 2 (15–20), 3 (10), 4 (5–10), >4 (up to 5). One cod, 37 cm long, had 10 *L. branchialis*. Larger cod, ca. 34–104 cm long, had only 1 copepod in most cases, rarely 2, and a maximum of 6 in a cod 52 cm long.

Sproston and Hartley (1941) found 1–4 *L. branchialis* on the final host *Merlangius merlangus* and 1–3 on a smaller number of *Pollachius pollachius*. Kabata (1958) says that 2 haddock were found each with 11 parasites of *L. obtusa*.

ADULT *L. branchialis* INFECTING OTHER FISHES THAN COD IN THE WESTERN ATLANTIC

Seventeen species other than the cod have been reported as final hosts of *L. branchialis* in the eastern Atlantic, including four species of the family Gadidae in addition to the cod as the only usual hosts (Kabata 1960). For western North Atlantic, in addition to the Atlantic cod, the Greenland cod, *G. ogac*, was reported as a final host at West Greenland (Wilson 1917) and from Newfoundland Labrador (Templeman and Fleming 1963). The Greenland cod lives much closer to shore from young to adult than the Atlantic cod. The rate of infection of *G. ogac* off Labrador by *L. branchialis* was extremely high and is important for understanding the reasons for the lower infection of cod in the same region.

Infection of *G. ogac* may vary considerably in different areas and samples. Off the wharf in 2-m depth at Domino, Labrador on July 15, 1948, of 19 *G. ogac* 19–25 cm long, 79% had the gill region infected with (average per infected fish 1.8) adult *L. branchialis*. At Spotted Islands near Domino, of several *G. ogac* from a cod trap set at 26-m depth, all had adult *L. branchialis* on the gills, some with 1–3 and one with 15 specimens. North of Hopedale, July 31–August 4, 1948, Greenland cod were jigged at Black Island and several other coastal localities but none possessed the copepod. At Nutak, Greenland cod examined on August 5–7, 1948 had no *L. branchialis* but on August 11, of 25 *G. ogac* caught in the harbor at Nutak, over depths of 18 m, 44% were infected with 1–6 (average per infected fish 2.2) adult *L. branchialis*.

An American plaice, *Hippoglossoides platessoides*, taken by otter trawl October 8, 1951 at 255 m in White Bay, Newfoundland, had one adult *L. branchialis* on its branchial arches. Two field technicians of the Biological Station reported that in the summer of 1953 a sculpin, either long-

horn or shorthorn, caught at Domino, Labrador had an adult *L. branchialis* attached in the usual position at the ventral anterior apex of the branchial arches.

In 1946–47 we examined large numbers of haddock from the Newfoundland banks for the presence of *L. branchialis* and none were found. However, Rojo (1959) found three *L. branchialis* on the branchial arches of 63 haddock from Division 3N and two on 141 haddock from Division 3O.

Acknowledgments

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Bacterial Flora of Aquarium Fishes and Their Shipping Waters Imported from Southeast Asia

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SHOTTS, E. B. JR., A. L. KLECKNER, J. B. GRATZEK, AND J. L. BLUE. 1976. Bacterial flora of aquarium fishes and their shipping waters imported from Southeast Asia. J. Fish. Res. Board Can. 33: 732–735.

Seventy-three plastic containers of aquarium fish and accompanying water from Taiwan, Singapore, Bangkok, and Hong Kong were studied to determine bacterial flora. It was found that 69% of the bags of fish contained individuals with bacteremias. When tissue suspensions of fish were examined 18 genera of bacteria were noted. Fourteen genera were found associated with the shipping water. In each case the two predominating organisms encountered were *Pseudomonas* sp. (not *aeruginosa*) and *Aeromonas hydrophila* complex. Other organisms found included *Citrobacter, Proteus, Escherichia, Micrococcus, Mycobacterium*, and *Flavobacterium*. Mycoplasmas were not recovered.

SHOTTS, E. B. JR., A. L. KLECKNER, J. B. GRATZEK, AND J. L. BLUE. 1976. Bacterial flora of aquarium fishes and their shipping waters imported from Southeast Asia. J. Fish. Res. Board Can. 33: 732–735.

Nous avons examiné 73 récipients en plastique de poissons d'aquarium et l'eau qu'ils contenaient en provenance de Taïwan, Singapour, Bangkok et Hong Kong en vue d'en déterminer la flore bactérienne. Nous avons découvert que 69% des sacs contenaient des individus affectés de bactérémies. Nous avons identifié 18 genres de bactéries dans les suspensions de tissu examinées. Quatorze genres étaient associés à l'eau d'expédition. Dans chaque cas, les deux organismes prédominants étaient *Pseudomonas* sp. (autre qu'*aeruginosa*) et le complexe *Aeromonas hydrophila*. Les autres organismes incluaient *Citrobacter, Proteus, Escherichia, Micrococcus, Mycobacterium* et *Flavobacterium*. Nous n'avons pas trouvé de mycoplasmas.

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FOR a number of years public health and other interested agencies have been involved in the microbiological examination of various pets sold through retail outlets as potential carriers of agents of human disease. The primary interest over the years has been on the salmonellae. Most recently, attention has been concentrated on the role of pet turtles as vectors of salmonellosis. Wells et al. (1973) stated that 300,000 of the estimated two million annual cases of human salmonellosis in the United States were probably associated with pet turtles.

Within the past year Trust and Bartlett (1974) have described the recovery of potential human pathogens from aquarium fish and their transport water as purchased from pet shops in Canada. The source of these organisms is of primary concern, particularly when one takes into account the fact that in excess of 20 million household aquaria are in operation in the United States at a given time (Axelrod 1973).

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During the past 18 mo, 16 shipments consisting of 73 lots of aquarium fish have been received for bacteriologic study in this laboratory. The fish were obtained through the auspices of the Pet Industry Joint Advisory Council of the United States (PIJAC). It was the purpose of this study to determine the bacterial flora associated with these fish and their accompanying shipping water prior to their introduction into pet shops in the United States.

Materials and Methods

SOURCE OF FISH

Sixteen shipments of aquarium fish were obtained from Southeast Asia over an 18-mo period. In most cases shipments of fish were forwarded to us within 24 h of receipt by the importer. Predetermined points of origin were Taiwan, Singapore, Bangkok, and Hong Kong, and four shipments were studied from each of these sources. Shipments usually contained five lots (bags) of approximately 50 fish each. Shipment was made from point of origin to a wholesaler who reoxygenated the shipping water and forwarded

the shipment via air express to this laboratory. Upon arrival the fish and their shipping water were systematically processed to determine the bacterial flora.

Media for Primary Isolation

Commercially available media, prepared according to the manufacturer's recommendations were used when possible. Media not commercially available were prepared according to directions found in the cited literature. Cultures were prepared in duplicate and incubated at both 23 and 35 C. Representative colonies were transferred to trypticase soy agar (TSA) and the organisms identified (Breed et al. 1957). Care was taken to select colonies representing all morphologic types noted.

Examination for Bacteremia

After the individual lots of fish were opened and a sample of five fish removed for parasitological studies, five of the remaining fish in each lot were randomly selected and cultured for evidence of bacteremia. This was accomplished by killing the fish, decontaminating the exterior surface of the fish by dipping in ethyl alcohol and igniting. The fish was aseptically dissected to expose the kidney for culture. In smaller fish (3 cm or less in length) often a mixture of blood and kidney was cultured. Cultures were streaked to both blood agar (BA) and Cytophaga agar (CA) plates (Anacker and Ordal 1959) for study.

Examination of Fish Tissue Slurry

Additional fish were killed, surface sterilized as described above, and homogenized without evisceration in a blender with sterile phosphate buffered saline (PBS) of pH 7.2 to achieve a 10% wt/vol suspension of fish. This slurry of tissue was divided, a portion being referred for virus isolation; the remainder was used to inoculate a battery of bacteriological media which were selected to insure recovery of a wide variety of microorganisms, should they be present. Selective media were used where possible to increase the probability of recovering specific organisms which might be present in small numbers.

The media used were: BA, general medium for detection of more fastidious organisms as well as many common organisms; CA, for growth of myxobacteria; TSA, used as a second general growth medium; Rimler–Shotts agar (RS) (Shotts and Rimler 1973), used to select members of the *Aeromonas hydrophila* complex and other enteric organisms; bismuth sulfide agar (BiS), used specifically to detect presence of *Salmonella typhi*. Tubes of selenite-brilliant green broth (SBG) and ducitol selenite broth (DS) (Raj 1969) were prepared in 2× concentration, an equal amount of slurry was added, and tubes were incubated at 41.5 C for 48 h with streaking to brilliant green agar (BGA) at 24 and 48 h to detect salmonellae.

Midway in the study, beginning with the ninth shipment and thereafter, tissue slurries were also tested for the presence of mycoplasmas and *Mycobacteria*. Slurries for these procedures were subjected to digestion with pancreatin (Oxoid Manual 1971). The mixture was then diluted with several volumes of PBS (pH 7.2), divided into four portions, and each run through a sterile Millipore filter unit equipped with a prefilter and a 0.45-μm disc. The filtrate was cultured in duplicate on mycoplasma media (Hayflick 1965; Barile et al. 1973) and incubated at 20 and 37 C. All cultures were examined and transferred to mycoplasma broth and agar media at weekly intervals. Examination continued for 4 wk before cultures were discarded as negative. The filter discs were transferred to the surface of Middlebrook and Cohn 7-H-10 agar plates, two of which were incubated at 23 and two at 37 C. The growth on each plate was periodically checked for the presence of acid fast bacteria, and efforts were made to remove and grow in pure culture any confirmed acid fast organisms. All filter disc cultures were examined for at least 2 mo before being discarded as negative.

Shipping Water

Upon arrival of the shipment, and concurrent with the procedures mentioned above, water samples from each shipping bag were pooled to achieve approximately 1500 ml of water representative of the respective shipment. This sample was centrifuged 2488 × *g* for 20 min at 4 C. The supernatant was retained for virus studies and the sediment resuspended in approximately 100 ml of the supernate. This latter material served as inoculum for an additional battery of media, namely: MacConkey agar (MAC) for detection of gram negative bacteria; thiosulfate-citrate-bile salts-sucrose agar (TCBS) as a selective medium for *Vibrio cholera* and/or *Vibrio parahemolyticus*; trypticase soy agar containing 1.5% sodium chloride (TSA-NaCl) for detection of organisms requiring increased salt concentration; RS for the selection of members of the *Aeromonas hydrophilia* complex and other enteric organisms; certrimide agar (PA) as a selective medium for members of the genus *Pseudomonas*; BiS to aid in the detection of *Salmonella typhi*. Examination for other salmonellae was accomplished by inoculation of tubes of SBG and DS in 2× concentration with an equal amount of suspended sediment with subsequent incubation at 41.5 C for 48 h with streaking to BGA at 24 and 48 h.

Beginning with the ninth shipment about 500 ml of the pooled shipping water sample was removed for mycoplasmal and mycobacterial analysis. The water was divided into four portions, each of which was passed through a sterile Millipore filter apparatus equipped with a prefilter and a 0.45-μm disc. The filtrates and filter discs were cultured as described above for fish tissue slurry.

Results

Bacteremias

Bacteremia, as measured by the presence of bacteria recovered from the kidney and/or blood

TABLE 1. Relative frequency of microorganisms recovered from blood–kidney, cultures of fish tissue slurries, and transport water of aquarium fish imported from South east Asia.

Bacteria	Blood–kidney		Tissue slurries		Transport water	
	No.	%	No.	%	No.	%
Pseudomonas sp.	31	36.5	44	16.5	12	19.0
Aeromonas hydrophilia	9	10.6	40	15.0	9	14.3
Staphlococcus sp.	9	10.6	11	4.1	2	3.2
Flavobacterium sp.	8	9.4	12	4.5	2	3.2
Proteus vulgaris	6	7.0	31	11.7	7	11.1
Citrobacter sp.	5	5.9	19	7.1	7	11.1
Bacillus sp.	5	5.9	10	3.8	1	1.6
Micrococcus luteus	3	3.5	10	3.8	—	—
Enterobacter sp.	3	3.5	19	7.1	7	11.1
Escherichia sp.	2	2.4	18	6.8	5	8.0
Aeromonas shigelloides	2	2.4	5	1.9	1	1.6
Pseudomonas putrificans	1	1.2	13	4.9	1	1.6
Providencia sp.	1	1.2	17	6.4	4	6.3
alpha streptococcus	—	—	6	2.3	1	1.6
Proteus rettgeri	—	—	3	1.1	—	—
Proteus mirabilis	—	—	2	0.8	—	—
Penicillum sp.	—	—	1	0.4	—	—
Salmonella arizonae	—	—	1	0.4	1	1.6
Clostridium perfringens	—	—	1	0.4	—	—
Serratia sp.	—	—	1	0.4	—	—
Micrococcus roseus	—	—	1	0.4	—	—
Corynebacterium sp.	—	—	1	0.4	—	—
Klebsiella	—	—	—	—	2	3.2
Micrococcus sp.	—	—	—	—	1	1.6
Totals	85	100.0	266	100.0	63	10.0

of examined fish, was detected in 15 of the 16 shipments examined. The lone negative shipment was the initial one. It was found that 69% of the lots (bags) of fish contained individuals with bacteremia. The organisms associated with these bacteremias and their frequency are presented in Table 1. Organisms belonging to 11 different genera were identified. The two most frequently encountered were *Pseudomonas* sp. (37%) and the *Aeromonas hydrophila* complex (11%). The characteristics of the observed *Pseudomonas* sp. included a lack of pyocyanin production and failure to grow at 41.5 C.

The *Aeromonas hydrophila* complex was characteristically maltose positive, ornithine and lysine negative, and all produced gas from fermentative processes with the exception of four isolates that were anaerogenic. Other organisms encountered were predominantly gram negative. However, *Staphylococcus* (coagulase positive), as well as *Bacillus* sp. (not anthrax) and *Micrococcus luteus* were recovered on occasion.

FISH TISSUE SLURRIES

Bacteria of 18 different genera were observed associated with fish tissue slurries. (Table 1). The two most frequently noted were *Pseudomonas* sp. (17%) having the characteristics noted above, and *Aeromonas hydrophila* complex (15%). In this case no anaerogenic aeromonads were observed. Only one isolate of potential public health importance was made. A recovery of *Salmonella arizonae* was made from a lot (bag) of fish originating in Hong Kong.

No mycoplasmas were isolated from any of the fish tissue slurries examined. Acid fast bacteria were observed in the tissue slurry of shipment 11. They appeared in plate cultures incubated at 25 and 37 C; however, only two colonies could be found on either plate and they were overgrown with gram negative contaminants. Isolation in pure culture was not accomplished.

SHIPPING WATER

Bacterial flora associated with the shipping waters studied (Table 1) were not remarkable; 14 of the 16 shipments were studied. Culture of these waters resulted in the recovery of bacteria representing 14 genera, the 2 most frequent being *Pseudomonas* sp. (19%) and *Aeromonas hydrophila* complex (14%). These isolates had the characteristics described above, and six isolates of

anaerogenic *Aeromonas hydrophila* complex were encountered. A single isolation of *Salmonella arizonae* was made from a shipment of water originating in Bangkok.

No mycoplasmas were isolated from any of the shipping water samples examined. Acid fast bacteria were observed in direct smears made from the filter cultures from shipments 10, 11, 14, and 16. In shipments 10 and 11 acid fast organisms were found at 25 and 37 C; however, in 14 and 16 they were noted only at 25 C. In all instances only a very few colonies were detected, and they were invariably overgrown with contaminants. Isolation in pure culture was not accomplished.

Discussion

A marked identity was found among the predominating organisms encountered in each segment of the study. The two most frequent organisms in all segments of the study were *Pseudomonas* sp. and *Aeromonas hydrophila* complex. *Citrobacter* spp. were noted as indicated from all sources. Speciation was not done on this group; however, isolates were sometimes recovered which presumptively grouped as *Salmonella* sp. on the basis of a routine battery of biochemicals which included ducitol, urea, and malonate. They further agglutinated with *Salmonella* polyvalent antiserum and *Salmonella* group C_1 antiserum. These isolates did not react in Spicer–Edwards *Salmonella* "H" (flaglar) antisera and grew when transferred to KCN broth. A similar isolate was reported by Trust and Bartlett (1974) in their report. These *Citrobacter* isolates could very easily be confused with salmonellae.

Isolations of *Enterobacter* sp., *Escherichia* sp., and *Proteus vulgaris* were frequently made during this study. Although no quantitative studies were done, the predominant flora encountered were gram negative rods. Procedures followed in these investigations should have allowed for the recovery of *Salmonella, Edwardsiella, Vibrio* sp., and *Pseudomonas aeruginosa.* The isolation of *Salmonella arizonae* lends credibility to the recovery techniques.

Since no mycoplasmas were found in any of the fish tissue or water samples tested, one might conclude that these organisms play only a minor, if any, role in Southeast Asian aquarium fishes, and that transmission of mycoplasmas via shipping water is apparently of negligible significance.

The results of these studies indicate that at the time aquarium fish are shipped from the wholesaler to commercial outlets their flora do not differ significantly from those present in fish under natural conditions, or in pond-raised food fish. The variety of bacteria isolated closely reflects those reported by Collins (1970) in a study of healthy fish and water from an oligotrophic lake in England. The variations noted may readily be associated with pond fertilization and other practices characteristic of intensive fish culture operations. Other data regarding the bacteria associated with aquarium fish (Trust and Bartlett 1974) were obtained under different conditions (retail pet stores) and vary somewhat from the findings of this study.

It would be presumptuous to assume from our findings that aquarium fish, or their transport water, present a source of potential disease to humans, especially as no widespread outbreaks of human disease have been directly associated with aquaria. Aquarium fish represent a unique group of pets, since direct human contact would be detrimental to them. However, common hygiene practices should be maintained in any relationship between man and pet animals, to minimize the exchange of their bacterial flora.

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Occurrence of R Factors Associated with *Aeromonas hydrophila* Isolates from Aquarium Fish and Waters

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SHOTTS, E. B. JR., V. L. VANDERWORK, AND L. M. CAMPBELL. 1976. Occurrence of R factors associated with *Aeromonas hydrophila* isolates from aquarium fish and waters. J. Fish. Res. Board Can. 33: 736–740.

Isolates obtained from aquarium fish and aquarium waters identified as belonging to the *Aeromonas hydrophila* complex were examined for the presence of R factors imparting conjugal transmissibility of antibiotic resistance to tetracycline and/or sulfa drugs. It was found that 25.5% of the isolates from fish which were resistant to one or more of the screening antibiotics and similarily 14.1% of the isolates from aquarium water carried such R factors. These studies suggest that the prophylactic use of tetracycline may be a predisposing factor in the emergence of R factor-carrying strains of bacteria in aquarium water.

SHOTTS, E. B. JR., V. L. VANDERWORK, AND L. M. CAMPBELL. 1976. Occurrence of R factors associated with *Aeromonas hydrophila* isolates from aquarium fish and waters. J. Fish. Res. Board Can. 33: 736–740.

Nous avons examiné des isolats provenant de poissons et d'eaux d'aquarium et identifiés comme appartenant au complexe *Aeromonas hydrophila*, en vue d'y déceler la présence de facteurs R impartissant une transmissibilité conjugale de résistance antibiotique à la tétracycline et/ou aux sulfamides. Nous avons constaté que 25.5% des isolats de poisson et 14.1% des isolats d'eau d'aquarium portaient de ces facteurs R. Ces étudent laissent entendre que l'usage prophylactique de la tétracycline peut être un facteur prédisposant à l'apparition de lignées de bactéries porteuses de facteurs R dans les eaux d'aquarium.

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EXTRACHROMOSOMAL infectious drug resistance was first noted in Japan in 1959 when multiple drug resistance was transferred between *Escherichia coli* and *Shigella* (Ochiai et al. 1959). Japanese investigators have studied the occurrence of R factors in gram-negative bacteria associated with fish and fish culture in Japan (Aoki et al. 1971a, b, 1972, 1973). Little information is available regarding the occurrence of R factors in bacteria associated with fish in the United States. R factor carriage has been reported from a single strain of *Aeromonas salmonicida* isolated in this country by S. F. Snieszko (Aoki et al. 1971a).

The prolonged use of antibiotics and other synthetic chemotherapeutic drugs as prophylactic agents to prevent disease, or as feed additives to promote weight gain, drastically increases the number of gram negative organisms carrying R factors in food-producing animals and fish (Smith 1969; Watanabe et al. 1971). The increased popularity of home aquaria in conjunction with the widespread use of tetracyclines as treatments for bacterial diseases of ornamental fish prompted us to determine the rate of R factor emergence in aeromonads associated with aquaria. Our concern was limited primarily to R factor carriage by isolates of the *A. hydrophila* complex (which include all mesophilic aeromonads currently listed in Bergeys except for *A. proteolytica*) because these organisms commonly found in surface waters may also be pathogenic for fish. Our interest was further limited to resistance to tetracycline and/or sulfa drugs since these are commonly used to treat disease conditions in fish.

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Materials and Methods

Isolates of *A. hydrophila* complex from aquarium fish were obtained during a study of the normal flora of common species of fish imported from Southeast Asia for sale in pet shops (Shotts et al. 1976).

Isolates of *A. hydrophila* complex from water were obtained from aquaria in two local pet shops. Shop I used tetracycline prophylactically; Shop II did not use drugs except for specific disease problems.

TABLE 1. Antibiogram patterns of drug resistance and their R factor transferability in isolates of the *Aeromonas hydrophila* complex from Southeast Asian aquarium fishes.

No. of resistance markers	No. of isolates	Resistant pattern[a]	No. of isolates	No. of isolates with R factor	Resistance[b] pattern of R factor
7	12	TE, ST, P, AM, DS, C, F/M	12	11	8TE, AM, DS, C, ST 3TE, DS, C, ST
5	9	TE, ST, P, AM, DS	4	0	
		TE, ST, AM, DS, C	1	1	TE, ST
		TE, ST, P, AM, C	3	0	
		ST, P, AM, DS, F/M	1	0	
4	34	TE, ST, P, AM	13	6	1TE, ST, AM 5TE, ST
		TE, ST, AM, C	1	1	TE, ST
		TE, ST, AM, DS	5	3	2TE, ST 1TE, ST, AM
		ST, P, AM, DS	11	0	
		ST, AM, DS, C	4	0	
3	32	TE, ST, AM	7	6	5TE, ST, 1TE, ST, AM
		TE, P, AM	1	0	
		ST, P, AM	23	0	
		P, AM, DS	1	0	
2	6	P, AM	3	0	
		P, ST	3	0	
1	17	ST	4	0	
		F/M	2	0	
		AM	10	0	
		P	1	0	
Total	110		110	28	(25.5%)

[a]Abbreviations: TE, tetracycline; ST, sulfathiazol; P, penicillin; AM, ampicillin; DS, streptomycin; C, chloromycetin; F/M, furadantin.

[b]Representative cultures confirmed in Department of Microbiology, Keio University School of Medicine, Tokyo, Japan by Drs Arai and Aoki.

Aquarium water was sampled by dipping water from aquaria using sterile plastic bags. Samples were returned at ambient temperature to the laboratory where they were streaked on Rimler–Shotts (RS) agar (Shotts and Rimler 1973) for isolation and selection of colonies for study. Usually 3–5 characteristic colonies from each plate were transferred to triple sugar iron agar (TSI) for further study.

All isolates examined for the presence of R factor were identified as members of the *A. hydrophila* complex using the parameters of maltose fermentation (+), arginine dihydrolase (+), lysine decarboxylase (−), ornithine decarboxylase (−), and oxidase (+) activities. A total of 415 isolates were examined, 215 from fish and 200 from aquarium water. All cultures were screened initially for resistance to selected antibiotics. This was accomplished by inoculating on a plate of 5% bovine blood Mueller–Hinton agar with each isolate. Following inoculation, Sensi discs (Baltimore Biological Laboratories, Cockneyville, Md.) of streptomycin (DS — 10 μg), sulfathiazole (ST — 1 mg), penicillin (P — 10 units), ampicillin (AM — 10 μg), tetracycline (TE — 30 μg), chloromycetin (C — 30 μg), furadantin (F/M — 300 μg), and naladixic acid (NA — 30 μg) were placed on each plate with subsequent incubation at 37 C for 24 h prior to interpretation. Resistance was recorded when no zone of inhibition was noted around a respective disc. Only isolates demonstrating resistance to one or more antibiotics were retained for further study.

All antibiotic-resistant isolates were transferred to Penassay broth (Difco) and incubated for 24 h at 37 C. The recipient organism used in this study was *Escherichia coli* 1932 (CDC) that was lactose negative, naladixic acid- and penicillin-resistant, but sensitive to the remaining antibiotics employed. This organism was transferred to a flask containing 250 ml of Penassay broth and incubated as above. Following incubation, equal amounts (1.0 ml) of cultures of the respective isolate and of *E. coli* 1932 were added to sterile tubes and reincubated at 37 C overnight. The following day these mixed cultures were streaked to MacConkey agar plates containing 30 μg ml^{-1} each of naladixic acid and tetracycline. These plates were incubated at 37 C overnight and 2–3 colonies of the resulting growth were transferred to TSI for characterization and subsequent antibiograms. The antibiotic resistance of each isolate attributed to R factor association was determined by

TABLE 2. Antibiogram patterns of drug resistance and their R factor transferability in isolates of the *A. hydrophila* complex from aquarium water in Pet Shops I[a] and II[b].

No. of resistance markers	No. of isolates	Resistance pattern[c]	No. of isolates	No. of isolates with R factor	Resistance[d] pattern of R factor
Shop I					
6	4	TE, ST, P, AM, DS, C	4	2	TE, AM, DS, C, ST
5	22	TE, ST, P, AM, DS	16	2	TE, ST, AM
		TE, ST, DS, AM, C	5	1	TE, ST, AM
		TE, ST, P, AM, C	1	1	TE, ST
4	44	TE, ST, P, AM	30	4	TE, ST, AM
		TE, ST, AM, DS	14	2	TE, ST
3	28	TE, ST, AM	13	3	TE, ST, AM
		TE, ST, DS	2	2	TE, ST
		ST, DS, AM	3	0	
		ST, P, AM	9	0	
		P, AM, DS	1	0	
2	17	ST, AM	8	0	
		ST, DS	1	0	
		ST, P	1	0	
		TE, AM	1	0	
		P, AM	6	0	
1	5	AM	4	0	
		P	1	0	
Total	120		120	17	(14.1%)
Shop II					
6	1	TE, DS, ST, AM, P, F/M	1	0	
5	13	TE, ST, AM, P, F/M	3	0	
		TE, DS, ST, AM, P	10	0	
4	13	TE, ST, AM, P	13	0	
Total	27		27		(0%)

[a]Shop routinely added 250 mg of tetracycline per 20-gal (76 liters) aquarium.

[b]Shop used antibiotics only in treatment of disease problems when encountered in fish care.

[c]Abbreviations: TE, tetracycline; ST, sulfathiazole; P, penicillin; AM, ampicillin; DS, streptomycin; C, chloromycetin.

[d]Representative cultures confirmed in Department of Microbiology, Keio University School of Medicine, Tokyo, Japan by Drs Arai and Aoki.

comparison of the antibiograms of each recombinant and *E. coli* 1932.

Results

It was found that 110 of the 215 isolates of *A. hydrophila* complex isolated from Southeast Asian aquarium fishes were resistant to one or more of the antibiotics used for screening. The numbers of resistant markers associated with isolates in this group varied from 1 to 7 (Table 1). The majority (66 of 110 or 60%) of the isolates had from 3 to 4 resistant markers and accounted for 16 of 28 (57.2%) R factors noted in this group. The remaining isolates demonstrating R factor carriage had from 5 to 7 resistant markers. Two incompatibility groups of R factors were encountered. The more prevalent group carried a resistance to tetracycline and sulfathiazole with an associated ampicillin resistance in some cases. These were considered as group A. The other R factor group noted carried a more involved resistance pattern which included tetracycline, streptomycin, chloromycetin, and sulfathiazole with an ampicillin resistance associated in some cases. These R factors were characterized as group N.

A total of 200 isolates of *A. hydrophila* complex from aquaria water was examined from Shops I and II. Among these isolates, 120 of those from Shop I (Table 2) and 27 from Shop II (Table 2) had resistant markers. The basic difference between the two shops was the prophylactic use of antibiotics (tetracycline) in Shop I. While antibiotic resistance was noted in bacterial isolates from both shops, only isolates from Shop I where tetracycline was used prophylactically, were demonstrated to carry conjugal R factors.

A majority of these isolates (Shop I) had either 4 or 5 resistant markers (77 of 120 or

64%). This same group contained 10 of 17 (59%) of the R factors noted in the water samples. All of these R factors imparted a resistance to tetracycline and sulfathiazole, and in some instances ampicillin resistance was noted. The isolates having 3 resistant markers contained five R factor-carrying strains, and all had resistance patterns similar to those mentioned above. The two remaining R factors noted in isolates from aquarium water were associated with isolates having 6 resistant markers. These R factors show transmissible resistance to tetracycline, ampicillin, streptomycin, chloromycetin, and sulfathiazole.

Discussion

Aquarium fish culture is widely employed in Southeast Asia. The potential for disease problems associated with such culture would increase the probability of the use of a number of drugs, including tetracycline, in their correction. It has been demonstrated repeatedly that the use of low levels of tetracycline either as prophylactic medication or for nutritional purposes may give rise to populations of tetracycline-resistant microorganisms. Most prominently affected are members of the Enterobacteraceae and related gram-negative rods (Smith 1973). These antibiotic resistant bacterial populations are thought to be the result of continuous low level antibiotic pressure on a population that possesses an exceptionally low mutation rate in vitro but mutate readily in vivo.

Resistance to tetracycline is probably one of the commonest if not the most common R factor associated resistance encountered. This perhaps is due to the location of this locus adjacent to the sex locus in the episomal DNA arrangement of the R factor (Davies and Rownd 1972).

Also, acriflavin, a chemotherapeutic agent commonly used in the fish industry, can eliminate R factors at certain concentrations but at other concentrations enhances the selection of acriflavin-resistant R factors (Brinton 1971; Arai and Watanabe 1967). These and other conditions may enhance the selection and growth of R factor-carrying strains of the *A. hydrophila* complex in the flora of aquarium fish available for sale.

Adverse environmental changes and other stresses in the handling of aquarium fish during shipment often result in the fish developing a bacteremia or septicemia (Shotts et al. 1976). It is a practice in some retail pet shops to treat the fish prophylactically with antibiotics upon arrival. If tetracycline is used, this antibiotic would aid in the selection of R factor-resistant strains of *A. hydrophila*.

We believe that situations such as those mentioned above contribute measurably as underlying causes responsible for the recovery of the R factor-carrying organisms cultured in our studies. The initial introduction of an antibiotic-resistant bacterial population in fish is of very little or no consequence since it may occur at a low frequency in the total bacterial population in the absence of antibiotics (Smith 1973). The potential danger that exists lies in the indiscriminant use of antibiotics, particularly tetracycline, as "cure-alls." This misuse of antibiotics may result in the selection of R factor-carrying clones of *A. hydrophila*. Ultimately an antibiotic-resistant bacterial population may result in a fish disease problem that would not respond to usual antibiotic therapy. This same bacterial population may also serve as a source of potential transfer of antibiotic resistance to other similar microorganisms.

There is reason to conclude from these observations that aquarium fish introduced into new environments should be held in isolated aquaria and allowed to acclimate to the existing conditions in the absence of prophylactic antibiotic therapy. Cautious and responsible use of antibiotics in aquarium fishes will aid in minimizing the development and spread of potential R factor-carrying organisms that may confer antibiotic resistance to otherwise antibiotic-sensitive bacterial species.

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Salinity Effects on Plasma Electrolytes of Channel Catfish, *Ictalurus punctatus*

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DAVIS, K. B., AND B. A. SIMCO. 1976. Salinity effects on plasma electrolytes of channel catfish, *Ictalurus punctatus*. J. Fish. Res. Board Can. 33: 741–746.

Plasma sodium and chloride levels of channel catfish (*Ictalurus punctatus*) increased after a 5-day exposure to 10 and 12 g/liter sodium chloride. Exposure of fish to 4 and 8 g/liter for 5 days resulted in plasma electrolyte levels higher than those of fish kept in aged tap water, but were not different from values usually found in freshwater fish. These observed differences may have been due to handling. Tests were performed during several months and no effect due to season was observed.

When fish in July (27 C) were exposed to 10 g/liter sodium chloride, the plasma electrolyte concentration increased for 48 h and then stabilized. Exposure to the same concentration in March (9 C) resulted in a slow increase of plasma electrolytes throughout the 13 days of the experiment. Injection of 100 μg/kg cortisol had no effect on plasma electrolyte increase during 48 h after transfer to 12 g/liter sodium chloride.

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Les niveaux de sodium et de chlorure plasmiques de la barbue de rivière (*Ictalurus punctatus*) augmentent après exposition de 5 jours à des concentrations de chlorure de sodium de 10 et 12 g/litre. L'exposition à 4 et 8 g/litre résulte en des niveaux d'électrolytes plasmiques plus élevés que ceux de poissons maintenus dans de l'eau de robinet vieillie. Cependant, les poissons maintenus dans l'eau de robinet vieillie lors de ces essais ont des niveaux d'électrolytes plus bas que ceux normalement observés en eau douce. Ces bas niveaux peuvent être le résultat de la manipulation. On a poursuivi ces essais pendant plusieurs mois et on n'a observé aucun effet dû aux saisons.

Quand des poissons sont exposés en juillet (27 C) à 10 g/litre de chlorure de sodium, la teneur en électrolytes plasmiques augmente pendant 48 h et se stabilise par la suite. L'exposition à 10 g/litre de chlorure de sodium en mars (9 C) entraine une augmentation d'électrolytes plasmiques plus lente mais de plus longue durée qu'en juillet, et qui se poursuit encore 13 jours plus tard. L'injection de 100 μg/kg de cortisol n'affecte pas les électrolytes plasmiques durant les premières 48 h après transfert dans 12 g/litre de chlorure de sodium.

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CHANNEL catfish, *Ictalurus punctatus*, occur primarily in fresh water but are found in brackish water in southern Louisiana (Perry 1968). Adult catfish are stenohaline freshwater fish that tolerate salt concentrations up to 11.5 g/liter of dilute sea water (Allen and Avault 1969), artificial sea water (Stickney and Simco 1971), or sodium chloride solutions in our laboratory. Acclimation increases this tolerance by only 0.5 g/liter (Allen and Avault 1969).

Limited information on plasma electrolyte concentrations of channel catfish in fresh water or on stenohaline freshwater fish in salt water is available. Stickney (1971) found no plasma electrolyte differences due to body weight nor between groups of channel catfish held at different densities in fresh water at various flow rates. When channel catfish were exposed to 235 mosmol/liter sodium chloride, the plasma osmolarity increased slightly after 22 h, then decreased to fresh water levels by 94 h (Lewis and Lewis 1971). In goldfish, which are also stenohaline freshwater fish, plasma sodium concentrations increased significantly from 142 meq/liter in fresh water to 188 meq/liter after a 4-day exposure to 190 mM sodium chloride (Ellory et al. 1972).

Most experimental data on saltwater tolerance have been reported from fish held at constant temperature conditions, and without regard to the season. In general, low temperatures tend to change plasma electrolyte concentrations toward that of the ion level in the medium; i.e. plasma electrolyte levels increase in salt water and decrease in fresh water. Plasma chloride and osmolarity in channel catfish (Block 1974) and plasma

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sodium in brown bullhead catfish (Grigg 1969) in fresh water decreased when the fish were transferred from a warm acclimation temperature to cold water. Carp (Wikgren 1953; Houston and Madden 1968) and goldfish (Houston 1962) have excessive plasma ion loss in fresh water at low temperatures. Although serum chemistry was not significantly different from 20 to 10 C, a 2-day exposure to 0.5 C resulted in a sharp decrease in plasma sodium and, after 18 days, a decrease in plasma chloride in brown bullhead catfish (Umminger 1971). No data is available on the effect of low temperature on freshwater stenohaline fish in salt water; however, in a number of saltwater fish low temperatures result in an increase of plasma electrolyte concentrations (Holmes and Donaldson 1969; Umminger and Kenkel 1975).

Plasma sodium, potassium, and chloride were lower in summer than in fall and early winter in the white sucker (Hickman 1964), and a decrease of plasma sodium in June and July has been described in alewives (Stanley 1969) and smallmouth bass (Shell 1959). These changes are not those that would be predicted due to temperature effects alone, and may reflect seasonal physiological differences in the osmoregulatory mechanism.

Transfer of freshwater fish to salt water represents a stress which may activate an adrenal steroid response. Adrenal steroid hormones have been shown to increase in euryhaline eels after transfer to salt water (Hirano 1969; Ball et al. 1971), and have been implicated in mediating leucocytosis of cold stress (Ball and Slicher 1962; Slicher et al. 1962). Adrenal secretion may represent a nonspecific stress response or a hormonal adjustment of the fish to stimulate electrolyte secretion from the body. Injections of cortisol decreased plasma sodium in fresh water rainbow trout (Holmes and Butler 1963) and goldfish (Umminger and Gist 1973) and restored intestinal sodium transport in hypophysectomized goldfish (Porthe-Nibelle and Lahlou 1975).

The objectives of this study were to determine the effects of salt water concentration, temperature, season, and exogenous cortisol on the plasma electrolytes of channel catfish. These data will contribute to the understanding of plasma electrolyte regulation of a stenohaline freshwater fish in salt water.

Materials and Methods

All fish used in these experiments were supplied by the Fish Farming Experimental Station, Stuttgart, Arkansas. Blood was obtained from unanesthetized animals by puncture of the caudal vessel and each fish was bled only one time. Lithium heparin was used as an anticoagulant. Plasma chloride levels were determined on a 50-μl sample with a Buchelor–Cotlove automatic titrator. Plasma sodium concentrations were measured with a Beckman DU-2 Spectrophotometer with a flame attachment. Percent plasma protein was read with an American Optical Goldberg Refractometer, and osmolarity was determined by freezing-point depression (Model 3L Osmometer, Advanced Instruments). Water samples were analyzed with these same instruments.

Seasonal Study

Five separate 5-day experiments were performed in March, April, June, July, and October 1971, to determine whether or not the response to salt water was affected by season. In each experiment, five groups of fish were placed in sodium chloride solutions mixed by weight in aged tap water to give the following concentrations: 0, 4, 8, 10, and 12 g/liter. Fish were transferred directly from fresh water to the test salt concentrations. A constant air supply was provided and the fish were not fed during the experiments. The fish were held indoors but on a natural photoperiod and at outdoor temperatures.

In the March and April studies, two fish (15–20 cm) were kept in each of four 3-liter jars. The medium was changed if it became clouded. In June, July, and October eight fish (20–25 cm) were placed in five different 66-liter aquaria. After 5 days, blood was drawn and analyzed.

Rate of Change in Plasma Electrolytes

Thirteen-day experiments were performed in March and June to determine the rate of electrolyte change in fish transferred from fresh water to hypertonic salt water (10 g/liter). The temperature averaged 9 C in March and 27 C in June, and the photoperiod was natural in both experiments. The fish were held in a filtered 830-liter aquarium and were fed a maintenance diet. Blood samples were drawn at various intervals for the duration of the experiment.

Effect of Cortisol on Electrolyte Alterations

Electrolyte changes were measured on 42 fish (average weight, 600 g) introduced into a 1300-liter tank containing 12 g/liter sodium chloride at 23 ± 1 C. At the time of transfer from fresh water half were injected intraperitoneally with fish saline (0.65% NaCl), and half were injected with cortisol (100 μg/kg) to evaluate its effect on plasma electrolytes. Blood samples were taken from six fish from each group every 6 h for 48 h. In addition, initial blood samples were taken from six fish not transferred to salt water.

Results

In general, plasma sodium and chloride levels were similarly affected by increased salinity over

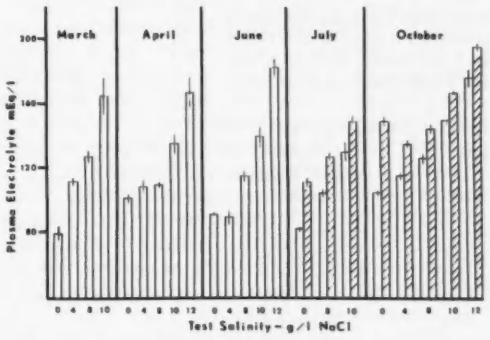

FIG. 1. Plasma chloride (open column) and sodium (hatched column) of channel catfish (*Ictalurus punctatus*) after a 5-day exposure to several concentrations of sodium chloride at different times of the year. Each column represents the mean of at least six fish ± SE.

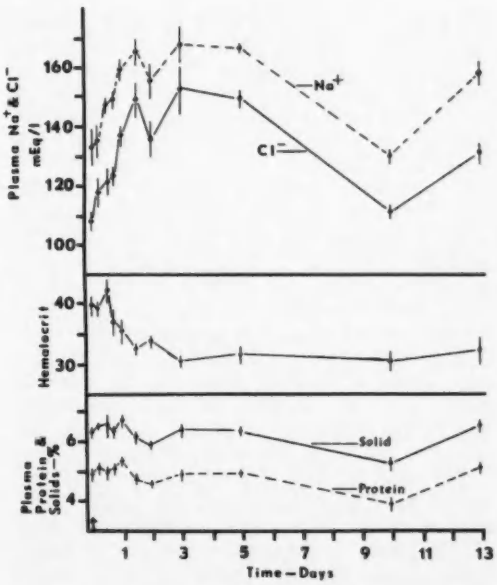

FIG. 2. Plasma sodium and chloride, percent solids and proteins, and hematocrit in channel catfish after transfer from fresh water to 10 g/liter sodium chloride in July (average monthly temperature 23 C). Time of transfer is indicated by the arrow. Each value represents the mean of at least six fish ± SE.

the five-day exposure (Fig. 1). In all tests, significant increases were measured in plasma sodium (July and October) and chloride (March, April, June, July, and October) of fish exposed to salinities of 10–12 g/liter. A salinity of 8 g/liter

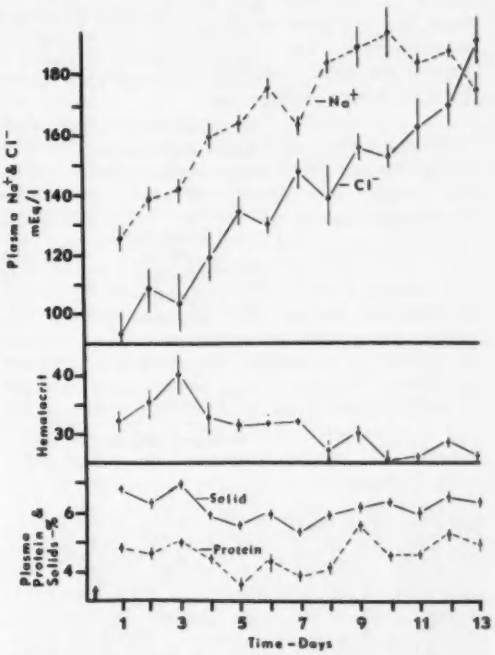

FIG. 3. Plasma sodium and chloride, percent solids and protein, and hematocrit in channel catfish after transfer from fresh water to 10 g/liter sodium chloride in March (average monthly temperature 9 C). Time of transfer is indicated by the arrow. Each value represents the mean of at least six fish ± SE.

resulted in significant increases in plasma chloride except in June, and plasma sodium in October but not July. In sodium chloride concentrations of 4 g/liter plasma chloride levels in three of the five tests (March, July, and October) and plasma sodium levels in July but not October were higher than those electrolytes from fish held in aged tap water.

In fish exposed to 10 g/liter NaCl in July, plasma sodium and chloride increased linearly through the first 48 h, then remained high through day 5 (Fig. 2). The sodium and chloride concentrations were elevated about 22, and 40%, respectively, by 36 h. The Na:Cl ratio remained relatively constant. Electrolyte levels were reduced on day 10 but were again elevated by day 13. Hematocrit fell and remained depressed from day one until the end of the experiment. Plasma solids and protein changes paralleled each other, and after a 1-day increase returned to levels similar to fresh water values.

In March, exposure of fish to 10 g/liter resulted in an increasing elevation in plasma sodium

TABLE 1. The effect of injections of cortisol (100 μg/kg) on plasma chloride and osmotic pressure increase upon transfer of channel catfish (*Ictalurus punctatus*) from fresh water into 12 g/liter sodium chloride.[a] Each value represents the mean of six animals + SE.

Hours after injection and introduction into salt water		Plasma Cl^- (*meq/liter*)	Plasma osmotic pressure (*mosmol/liter*)
0	Fresh water	119.8±1.2	279±2.7
6	Control	134.4±1.6	307±5.6
	Cortisol	131.2±0.8	302±4.4
12	Control	138.9±1.3	320±2.0
	Cortisol	139.5±1.3	321±2.4
24	Control	147.8±2.0	333±3.6
	Cortisol	149.6±2.0	336±3.1
48	Control	180.7±2.7	394±3.8
	Cortisol	177.6±3.5	389±9.9

[a]Water characteristics: Cl^- = 208 meq/liter; π = 381 mosmol/liter; temperature = 23±1 C.

and chloride throughout the 13-day period (Fig. 3). The rate of increase was less but plasma electrolyte concentrations reached higher levels compared to the July experiment. Five days were required before the same percent increase occurred as that attained after 2 days in July. Plasma protein and solids were erratic throughout the experiment.

Cortisol injections had no effect on the increase of plasma chloride, osmotic pressure, or percent protein when fish were transferred from fresh water to 12 g/liter NaCl (Cl^- = 208 meq/liter; π = 380 mosmol/liter), at 24 ± 2 C. Both saline- and cortisol-injected fish had rapidly increasing plasma chloride and osmotic pressure through the 48 h (Table 1).

Discussion

Plasma electrolyte concentrations in the upper two test salinities (10 and 12 g/liter) were consistently elevated above values normally found in fish in fresh water. When goldfish are exposed to hypertonic salt solutions plasma electrolyte concentrations rise and become equal to or slightly higher than the external concentration (Lahlou et al. 1969; Ellory et al. 1971, 1972). Therefore, the tolerance to brackish water in channel catfish, and perhaps goldfish, probably reflects the ability of these fish to withstand increased osmotic pressure of internal fluids. However, a difference in the osmoregulatory capacity of goldfish and catfish is indicated, because goldfish require gradual adaptation to tolerate one-half strength sea water (Pora 1939; Lahlou 1967). The sea water tolerance of channel catfish can be increased only 0.5 g/liter (11.5–12.0 g/liter) by prior acclimation to lower salinities (Allen and Avault 1969).

Electrolyte concentrations from control fish in the 5-day tests in every case, except sodium in the October series, were lower than concentrations normally found in hundreds of fish held in fresh water in our lab (Cl^- = 115 meq/liter; Na^+ = 133 meq/liter). Stress of handling in salmon, trout (Wedemeyer 1972), and goldfish (Meyer 1948; Umminger and Gist 1973) and anoxia in goldfish (Meyer et al. 1956) caused a decrease in plasma sodium and chloride of fish held in fresh water. Therefore, the apparent differences between plasma electrolyte concentrations of the control and the two low salinities (4 and 8 g/liter) may reflect a handling effect on the controls rather than an elevation of the experimental groups. No difference in plasma electrolyte response to salt water was observed due to season.

The rapid increase of plasma electrolytes that occurs upon introduction into 10 g/liter NaCl requires at least 48 h at warm temperatures (July 27 C) before an elevated equilibrium is established. These changes appear to move much slower than the 48-h adjustment period, composed of an increasing and decreasing phase, described for eels and flounder transferred from fresh to sea water (Keys 1933; Lahlou 1967). In freshwater-acclimated steelhead trout, plasma chloride increased through the first 40 h, then decreased until 160 h after transfer from fresh to salt water (Houston 1959). Eel, flounder, and trout are euryhaline fish, and these experiments were conducted with fish transferred from fresh water to full strength sea water and at temperatures similar to the March experiment reported here. Carp, a

stenohaline freshwater fish, exposed to a 12 g/liter NaCl solution doubled tissue chloride in 5 h after transfer from fresh water (Takemura 1951). However, these fish were restricted in movement and were anesthetized with urethane; therefore, it is difficult to compare the present study with those on carp. When channel catfish were exposed to a 235 mosmol/liter (approximately 7 g/liter) NaCl solution, an increase in plasma osmotic pressure was observed after 22 and 46 h, but returned to freshwater levels by 94 h. Plasma osmotic pressure in golden shiners exposed to the same concentration increased linearly through 94 h (Lewis and Lewis 1971). Unfortunately, no statistical treatment of the data was reported.

In March, at a colder temperature (9 C), plasma electrolytes increased more slowly and continued to increase to levels much higher than those in July and the Na:Cl ratio fell below 1.0 by the 13th day. Plasma sodium and chloride concentrations were not significantly different from the medium concentrations by the end of the experiment. The fish remaining at the end of the March experiment exhibited signs of severe dehydration. The eyes were recessed, they were difficult to bleed, and a few died. In the July experiment a significant but apparently transient decrease in plasma electrolytes back to freshwater levels occurred. Furthermore, the Na:Cl ratio remained approximately 1.18, a ratio similar to that of freshwater catfish. Fish used here were comparable in size to the other species reported, so size could not explain the relatively slow increase of electrolytes in channel catfish exposed to 10 g/liter NaCl.

In goldfish (Meyer et al. 1956; Houston 1962; Prosser et al. 1970; Umminger and Gist 1973), carp (Houston and Madden 1968), brown bullhead (Grigg 1969; Umminger 1971), and channel catfish (Block 1974) held in fresh water and exposed to extremely low temperatures, serum electrolytes decreased. However, plasma electrolyte concentrations have been reported to be independent of temperatures from 10 to 20 C in goldfish (Umminger and Gist 1973) and brown bullhead (Umminger 1971), and this includes the temperature range reported here for channel catfish. Experimental evidence from goldfish transferred to salt water suggests that intestinal transport of sodium is decreased (Ellory et al. 1972), branchial sodium (Lahlou et al. 1969; Lahlou and Sawyer 1969) and chloride (DeRenzis and Maetz 1973) influx and outflux is increased, and urine electrolyte content is increased and urine volume decreases (Lahlou and Sawyer 1969). The slower increase of electrolytes observed in March was likely due to a decreased diffusion rate; however, the continued increase was likely due to a low metabolic rate resulting in the branchial and renal adjustments in permeability and ion movement not occurring rapidly enough to offset the inward movement of ions and outward movement of water from the body.

Stanley and Colby (1971) have shown a detrimental effect of low temperature on salt water osmoregulation in the alewife and suggest that electrolyte shifts induced by acute exposure to cold may be related to mass mortalities that have occurred in Lake Michigan (Stanley 1969). The March experiment presented here suggests that low temperatures may limit the salt tolerance of channel catfish. Decreased salt tolerance in colder months could limit the distribution of catfish in brackish water (Perry 1968) and the usefulness of brackish water for culturing channel catfish as suggested by Allen and Avault (1969).

No effect of cortisol injections on plasma chloride or osmotic pressure was observed during 48 h after transfer of catfish from fresh water to 12 g/liter NaCl. Plasma cortisol levels doubled when Japanese eels were transferred from fresh to sea water (Hirano 1969). Cortisol injections in goldfish in fresh water decreased serum electrolytes (Umminger and Gist 1973) and restored intestinal transport of sodium lost after hypophysectomy (Porthe-Nibelle and Lahlou 1975). Sodium turnover is dramatically affected by interrenalectomy and cortisol injections. Cortisol is capable of stimulating active transport of sodium in eels either into or out of the body (Maetz 1969). No measurements of sodium turnover were estimated in channel catfish; however, since no effect of cortisol injections were observed on plasma chloride or osmotic pressure, cortisol is apparently not involved in osmotic balance of channel catfish in salt water.

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Assessment of the United States Lake Whitefish (*Coregonus clupeaformis*) Fisheries of Lake Superior, Lake Michigan, and Lake Huron

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JENSEN, A. L. 1976. Assessment of the United States lake whitefish (*Coregonus clupeaformis*) fisheries of Lake Superior, Lake Michigan, and Lake Huron. J. Fish. Res. Board Can. 33: 747–759.

The logistic surplus production model is applied to lake whitefish (*Coregonus clupeaformis*) yield and effort data from Lake Superior, Lake Michigan, and Lake Huron. The fitted models indicate that the whitefish populations in most areas of the lakes have been overexploited, and that in these areas yield was below the maximum sustainable yield before sea lamprey (*Petromyzon marinus*) predation took its toll. In some areas of Lake Superior the whitefish populations are not overexploited. The sea lamprey is the apparent cause of the drastic decrease in the whitefish population that occurred in northern Lake Michigan during the 1950's.

JENSEN, A. L. 1976. Assessment of the United States lake whitefish (*Coregonus clupeaformis*) fisheries of Lake Superior, Lake Michigan, and Lake Huron. J. Fish. Res. Board Can. 33: 747–759.

L'auteur applique le modèle logistique de surplus de production aux données relatives au rendement et à l'effort de pêche du grand corégone (*Coregonus clupeaformis*) des lacs Supérieur, Michigan et Huron. Les applications du modèle indiquent que les populations de grands corégones dans la plupart des régions de ces lacs ont été surexploitées et que, dans ces régions, le rendement était inférieur au rendement maximum soutenu avant que la prédation par la lamproie marine (*Petromyzon marinus*) n'occasionne de mortalités. Dans certaines régions du lac Supérieur, les populations de grands corégones ne sont pas surexploitées. La lamproie marine semble être la cause du déclin dramatique de la population de grands corégones qui se produisit dans le lac Michigan septentrional durant les années 1950.

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IN recent years there have been several efforts to explain the changes that have occurred in the Great Lakes fisheries. The studies of Smith (1968) and Christie (1974) provide two comprehensive but somewhat different descriptions of the rise and fall of fish species of the Great Lakes. Smith (1968) considered overfishing and introduced species to be the major factors causing the deterioration of the Great Lakes fisheries. Christie (1974) thought that Smith had overemphasized the effects of fishing, and he considered introduced species such as the sea lamprey (*Petromyzon marinus*) to be the major factor causing the deterioration of the Great Lakes fisheries.

Few attempts have been made to apply yield equations to the Great Lakes fisheries. Sakagawa and Pycha (1971) applied Ricker's (1945, 1975) dynamic pool yield equation to study yield of the lake trout (*Salvelinus namaycush*) in Lake Superior prior to the depredations of the sea lamprey. Eshenroder and Haas (1974) and Keller et al. (1974) applied Ricker's model to study lake whitefish (*Coregonus clupeaformis*) in Lake Huron and Lake Michigan, respectively. These attempts to apply Ricker's model were limited by the lack of adequate data for separating natural and fishing mortality. Good estimates of recruitment also were unavailable.

Although data for application of dynamic pool models are limited, data on yield and effort for many species using different fishing gears have been collected since 1929 for Michigan waters of the Great Lakes and since 1949 for waters of Minnesota, Illinois, Indiana, and Wisconsin. Similar data have been successfully applied to construct surplus production yield equations for management of other fisheries (Schaefer 1954, 1957). Walter and Hogman (1971) applied a surplus production model to the whitefish fishery of statistical district MM-1 in northern Lake Michigan and concluded that the sea lamprey was more important than commercial fishing in causing the decline of this fishery.

In this study the logistic surplus production

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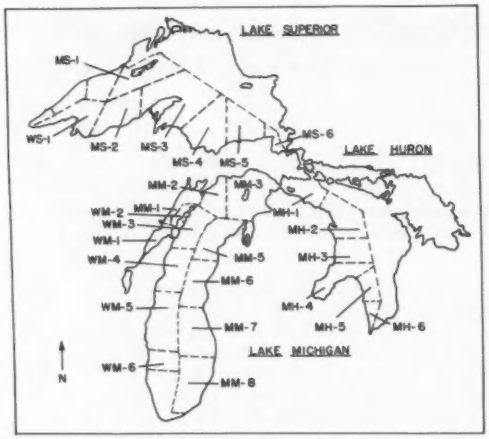

FIG. 1. Statistical districts of Lake Superior, Lake Huron, and Lake Michigan examined in this study.

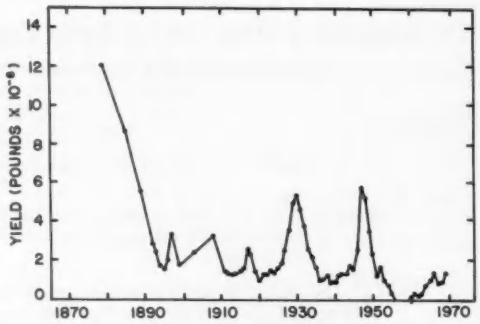

FIG. 2. Lake whitefish (*Coregonus clupeaformis*) yield from Lake Michigan from 1879 to 1970 (Wells and McLain 1973).

model is applied to the whitefish yield and effort data of selected districts of the United States waters of Lake Superior, Lake Huron, and Lake Michigan. The biological interpretation and management implications are investigated, and the carrying capacity, biomass levels, maximum sustainable yield, fishing effort that produces the maximum sustainable yield, and the relative importance of commercial fishing and sea lamprey predation are determined.

Summary of Yield and Effort Data

Yield and effort data are recorded for licensed commercial fishermen throughout the United States and Canadian waters of the Great Lakes. These data are tabulated by statistical districts which are described by Smith et al. (1961) and Hile (1962). The statistical districts included in this study are MS-1 through MS-6 in Lake Superior; MH-1 through MH-6 in Lake Huron; and WM-1 through WM-6, and MM-1 through MM-8 in Lake Michigan (Fig. 1). Minnesota statistical districts of Lake Superior have not been included because the data were not reported in the same form as the others and it was difficult to obtain the necessary yield and effort data. Indiana and Illinois have not been included because few whitefish are caught in these districts.

In nearly all districts the major gear used for whitefish was 4½-inch gillnets (11.43 cm). A lift of 1000 linear feet (300 m) of gillnet was defined by Hile (1962) as one unit of effort. Poundnets, deep trapnets, and shallow trapnets were also important gear for whitefish. A deep trapnet has the pot from 720 to 1200 cm deep or deeper, and a shallow trapnet has the pot less than 450 cm deep. One lift of a trapnet or poundnet is one unit of effort. The above definitions of a unit of effort do not consider the depth of gillnets, the size of poundnets and trapnets, whether gillnets are cotton or nylon, and the length of time that a net may be in the water. Hile (1962) reported that the length of time that a gillnet was in the water between lifts did not significantly affect yield per unit of effort.

Several different patterns appear in the yield and effort data. In Lake Superior yield is not large and is relatively constant from year to year (Fig. 16). In northern Lake Michigan yield has fluctuated regularly from high to low levels (Fig. 10). Yield from southern Lake Michigan is smaller than that in the north; northern Lake Huron also has a smaller yield than northern Lake Michigan. From 1954 to 1961 yield from northern Lake Huron decreased to nearly zero (Fig. 21).

Important changes occurred in the whitefish fishery before collection of yield and effort data began in 1929. For example, in Lake Michigan annual yield during the 1800s was several times higher than any annual yield that has been observed since 1929 (Fig. 2). The historical catch record for Lake Michigan indicates that after an initial decrease in yield, regular fluctuations occurred. Catch data are not available for the period of increasing yields that occurred during development of the whitefish fishery before 1879. Historical description of the whitefish fishery is difficult because only part of the necessary data are available.

To apply the logistic surplus production model, the yield and effort data are converted to a standard gear. Both 4½-inch gillnets and pound-

nets are used as standard gear and the results for these two gears are compared. The total yield per unit of effort (YPE) for each standard gear is calculated as

$$\text{YPE} = \text{Total Yield/Total Effort}$$

where Total Effort $= (x)(y)/z$

x = effort with standard gear
y = total yield
z = yield with the standard gear

Review of Logistic Surplus Production Model

Surplus production models for yield from a fish population assume that the latter has some natural capacity for increase which is some function of population size. If the annual yield of a fishery equals the annual rate of increase of a population, no change in population size will occur. Surplus production is the amount of biomass that can be removed by a fishery without changing the size of a stock. At both high and low levels of stock size surplus production is low. The maximum surplus production occurs at some intermediate population size. The equations of the surplus production model based on the logistic equation are:

$$\frac{dY}{dt} = qEB \tag{1}$$

$$\frac{dB}{dt} = kB - \frac{k}{K}B^2 - qEB \tag{2}$$

where Y = yield in kg
t = time in years
q = catchability coefficient
E = fishing effort
B = biomass of fish population in kg
k = population growth constant
K = environmental carrying capacity

The logistic surplus production model was developed by Hjort et al. (1933) and by Graham (1935).

To apply the logistic surplus production model to the Great Lakes whitefish populations several assumptions are necessary. These assumptions have been discussed in some detail by Pella and Tomlinson (1969). The logistic surplus production model is a deterministic model. The growth of populations and the interactions between fishermen and fish are not deterministic processes, but the complexity of stochastic models discourages their application. It is assumed that deterministic models describe the mean population size, and variation about this mean is expected. A comparison of the two models indicates that the mean values of stochastic processes are often identical to the values predicted by deterministic models (Bailey 1964). Exploited populations are large and deterministic surplus production models have been successfully applied to these populations.

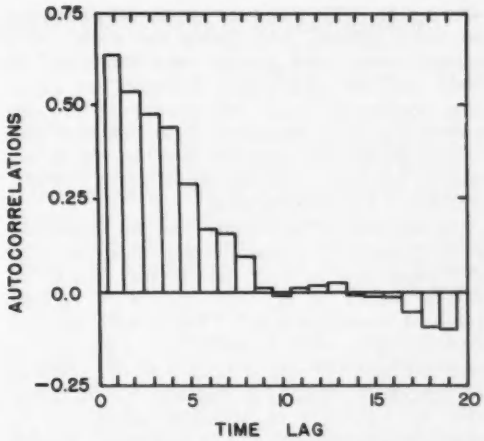

FIG. 3. Autocorrelation for whitefish yield per unit of effort in district MM-1.

Surplus production models ignore the age structure of a population, and are applied under steady state conditions; therefore, ignoring the age structure is equivalent to the assumption of a stable age distribution imposed by the constant birth and death rates. Populations to which surplus production models are applied are not in steady states; therefore, the observed catch and effort data will often fluctuate widely about the predicted equilibrium stock production curves.

The logistic surplus production model ignores time lags (e.g. a possible reproductive time lag), but a plot of the autocorrelation function for YPE indicates that such time lags are not important. The YPE autocorrelations decay to zero exponentially (Fig. 3) which indicates that the YPE data fit a first order autoregressive model of the form $(\text{YPE} - \mu)_{t+1} = \phi(\text{YPE} - \mu)_t + e_t$, where μ is the mean YPE, ϕ is a constant, and e_t is a random variable which is a function of time (Box and Jenkins 1970). The best predictor of biomass for year t is the biomass at year $t - 1$ and not the biomass at some year further in the past.

It is assumed that catchability remains constant, which is only approximately true. During the years when lake trout populations were low fishermen certainly changed their methods to concentrate more on whitefish. Also, catchability of gillnets may have changed from 1929 to 1973.

During the 1950s fishermen switched from cotton to nylon gillnets, and during the early 1960s monofilament nylon gillnets were introduced. In their study of the southern Georgian Bay whitefish population Cucin and Regier (1965) assumed that nylon gillnets were more efficient than cotton gillnets, but Hogman (1973) has shown that in northern Lake Michigan, district MM-1, this is not true for whitefish.

In this study it is assumed that the population in each district is a separate unit stock. Actually, the population in each district may consist of several stocks (S. H. Smith and A. T. Wright personal communication). The stocks are not entirely independent, however, and extinction or near extinction of a local stock does not result in an irreplacable loss as is indicated by the depletion and recovery of whitefish populations in northern Lake Michigan. As a first approximation it is reasonable to consider the entire population of each district as a separate stock; however, the identification of truly separate stocks must be of high priority for fishery research on the Great Lakes.

Finally, the logistic surplus production model assumes that the stock production curve is a parabola. This is the simplest possible realistic relation between stock and production, and for the whitefish fishery no other assumption is warranted. Given all of the above assumptions it is clear that the results must be interpreted carefully.

The catastrophic decrease in yield that occurred in Lake Michigan, which apparently was caused by the sea lamprey, could not be ignored, and a modified form of the logistic surplus production model that included lamprey predation was applied to the data of district MM-1 in northern Lake Michigan. The modified equations are

$$\frac{dY}{dt} = qEB \tag{3}$$

$$\frac{dB}{dt} = kB - \frac{k}{K}B^2 - qEB - pPB \tag{4}$$

where P is the lamprey abundance index (Walter and Hogman 1971) and p is a constant. All other terms in equations (3) and (4) are the same as those in equations (1) and (2).

Application of Surplus Production Models for Fishery Management

Surplus production models are applied to determine the maximum sustainable yield (MSY) and the level of fishing effort that will produce the MSY. The answers to these questions are necessary but not sufficient for management of a fishery.

In the steady state, $dB/dt = 0$, and annual equilibrium yield, Y_e, is given as

$$Y_e = kB - \frac{k}{K}B^2 \tag{5}$$

Equation (5) gives the annual equilibrium yield that will occur at each biomass level. Few fisheries are in equilibrium and observed yield and biomass values usually fluctuate considerably about the values predicted by equation (5) (the stock production curve).

To obtain the population size at which the MSY occurs, equation (5) is differentiated with respect to B; the result is set equal to zero and solved for B to give $B_1 = K/2$, where B_1 is the biomass at the MSY. The MSY is obtained by substitution of B_1 into equation (5)

$$MSY = kB_1 - \frac{k}{K}(B_1)^2 = \frac{kK}{4} \tag{6}$$

The fishing effort that gives the MSY is

$$E_1 = \frac{MSY}{(B_1)q} = \frac{k}{2q} \tag{7}$$

The fitted surplus production model can also be applied to calculate the biomass of the stock and the amount of biomass killed by the sea lamprey. Average biomass of the population during year t is estimated as $Y_t/(q\,E_t)$, where Y_t is the annual yield for year t and E_t is the effort applied during year t. Biomass of whitefish killed by sea lamprey during year t is estimated as $p\,P_tB_t$.

Economists have applied surplus production models to develop bioeconomic models for fisheries (e.g. Christy and Scott 1965). Bioeconomic models are applied to determine the maximum economic yield (MEY). The MEY is the yield that gives the largest sustainable economic rent. Under equilibrium conditions, if it is assumed that revenue, R, is proportional to yield, the equation for revenue as a function of effort or biomass is

$$R = h\left(kB - \frac{k}{K}B^2\right)$$

$$= h\left(qKE - \frac{Kq^2}{k}E^2\right) \tag{8}$$

where h is the revenue per unit of yield and R is total revenue. Equation (8) states that as fishing effort increases, revenue increases to a maximum and then decreases. If it is assumed that cost (C) is proportional to effort, then

$$C = gE = g\frac{Y_e}{qB} = \frac{gk}{q}\left(1 - \frac{B}{K}\right) \quad (9)$$

where g is the cost per unit of effort.

The MEY is determined from the revenue and cost equations. The economic rent, r, is the difference between the revenue and cost, i.e. from equations (8) and (9)

$$r = R - C$$

$$= h\left(kB - \frac{k}{K}B^2\right) - \frac{gk}{q}\left(1 - \frac{B}{K}\right) \quad (10)$$

Differentiation of equation (10) with respect to biomass, setting the result equal to zero, and then solving for biomass shows that the maximum economic rent and thus the MEY occurs at the biomass level

$$B^* = \frac{K}{2} + \frac{g}{2pq} = B_1 + \frac{g}{2pq} \quad (11)$$

The biomass at which the MEY occurs is larger than the biomass at which the MSY occurs; the MEY is smaller than the MSY.

An important result of the economic analysis of an open fishery is that the "law of the commons" predicts that such a fishery will operate at the point where economic rent equals zero, i.e. at the point where the cost curve and revenue curve intersect. This usually leads to overexploitation.

Estimation of Parameters

Accurate estimation of the parameters k, K, and q in equations (1) and (2) is difficult when only catch and effort data are available. Several different procedures have been applied. Schaefer (1957) developed a method for linearization of equation (2). From equation (1) average biomass during year t can be estimated as

$$B_t = (1/q)(Y_t/E_t) = (1/q)(U_t) \quad (12)$$

where Y_t is annual yield and U_t is yield per unit of effort for year t. The derivative in equation (2) can be approximated with the two point formula (Hamming 1962) which gives

$$\left(\frac{dB}{dt}\right)_t = \frac{B_{t+1} - B_{t-1}}{2} = (1/q)(\Delta U_t) \quad (13)$$

where $\Delta U_t = (U_{t+1} - U_{t-1})/2$. Substitution of equations (12) and (13) into equation (2) gives

$$\Delta U_t = kU_t - \frac{k}{Kq}U_t^2 - qY_t \quad (14)$$

and this can be written as the linear equation

$$Z_t = a_1X_1 + a_2X_2 + a_3X_3 \quad (15)$$

where: $Z_t = \Delta U_t$, $X_1 = U_t$, $X_2 = U_t^2$, $X_3 = Y_t$, $a_1 = k$, $a_2 = -k/Kq$, and $a_3 = -q$. The constants a_1, a_2, and a_3 can be estimated by least squares.

The derivative, dB/dt, in equation (2) cannot be estimated accurately using annual catch and effort data, and if yield and YPE are closely related, the determinant of the matrix $X'X$ may be zero or close to zero and accurate estimates of k, K, and q will not be obtainable.

Pella and Tomlinson (1969) developed a second method that uses numerical integration instead of approximation of the derivative. Equation (2) is a special case of Bernoulli's differential equation, and it can be solved in closed form. Substitution of the solution of equation (2) into equation (1) gives an equation for yield as a function of time that can be integrated numerically for given values of the parameters K, q, and k. To estimate these parameters initial values are guessed and equation (2) is integrated numerically; then a computer search routine is applied in which the initial guesses of the parameters are adjusted systematically over a bounded parameter space to find values of the parameters that minimize the residual sum of squares. This method works well when points on both sides of the maximum of the stock production curve are available (Pella and Tomlinson 1969).

Schaefer (1954) proposed a third method, an equilibrium approximation method, that has been further elaborated by Gulland (1969) and Fox (1975). For the surplus production model several equilibrium relations such as equation (16) can be derived, and these equilibrium relations are easily linearized. These methods may not work well, however, for fisheries that are never at equilibrium.

None of the above methods worked consistently for the whitefish. The procedure found to be most useful was to guess the values of the parameters and then adjust the guessed values to obtain a good fit on visual inspection of the stock production curve. Pella and Tomlinson (1969) give a method for making initial guesses of the parameters.

Relation between Yield and Effort

For all of the statistical districts the relation between yield and effort is described by the simple linear equation

$$Y_t = b\,E_t$$

TABLE 1. Yield per unit of effort, b, and correlation coefficient, r, between observed yield and yield calculated a $Y = bE$, where Y is yield and E is effort.

Districts	4½-inch Gillnet		Poundnets		Deep trap		Shallow trap	
	b	r	b	r	b	r	b	r
WS-1	8.22	0.69	52.84	0.87	—	—	—	—
MS-1	9.62	0.56	—	—	—	—	—	—
MS-2	10.97	0.82	93.44	0.67	—	—	—	—
MS-3	5.13	0.87	53.96	0.84	—	—	52.56	0.87
MS-4	6.64	0.26	68.69	0.88	—	—	72.70	0.44
MS-5	18.59	0.89	—	—	—	—	—	—
MS-6	6.86	0.36	57.23	0.84	—	—	78.46	0.77
MH-1	8.61	0.86	64.41	0.91	91.48	0.83	85.87	0.60
MH-2	3.95	0.84	75.76	0.95	116.20	0.92	—	—
MH-3	5.55	0.95	155.36	0.66	—	—	—	—
MH-4	7.48	0.97	37.37	0.91	169.29	0.64	4.83	0.17
MH-5	15.87	0.96	—	—	258.43	0.95	—	—
MH-6	10.52	0.96	246.59	0.47	237.04	0.75	8.52	0.88
WM-1	4.50	0.51	—	—	—	—	—	—
WM-2	4.61	0.67	96.13	0.66	—	—	—	—
WM-3	4.60	0.67	83.39	0.94	—	—	—	—
WM-4	—	—	—	—	—	—	—	—
WM-5	—	—	—	—	—	—	—	—
WM-6	—	—	—	—	—	—	—	—
MM-1	12.50	0.76	103.44	0.79	—	—	60.37	0.45
MM-2	4.82	0.64	—	—	—	—	—	—
MM-3	6.70	0.60	130.62	0.95	—	—	247.00	0.95
MM-4	3.58	0.72	62.37	0.93	—	—	—	—
MM-5	7.47	0.88	95.13	0.85	—	—	—	—
MM-6	5.82	0.93	—	—	—	—	—	—
MM-7	16.66	0.96	34.14	0.37	—	—	—	—
MM-8	14.51	0.77	—	—	—	—	—	—

where Y_t = annual yield during year t
E_t = effort during year t
b = constant, the regression estimate of yield per unit of effort

This was fitted to the major gears used in each district. The constants and the correlation coefficients between observed and predicted yields are listed in Table 1; the correlations are high.

The linear relation between yield and effort, together with the widely held assumption that fish stocks of the Great Lakes are overexploited, indicates that the fisheries are not at equilibrium. Under equilibrium conditions the relation between annual equilibrium yield, Y_e, and effort predicted by the logistic surplus production model is the parabola

$$Y_e = qKE - \frac{Kq^2}{k}E^2 \qquad (16)$$

Equation (16) predicts that as effort increases yield increases to a maximum and then decreases as the population becomes overexploited.

In the nonequilibrium condition the relation between annual yield, Y_t, and effort at each level of biomass is the straight line

$$Y_t = qE\int_0^1 B\,dt = bE \qquad (17)$$

which is obtained from equation (1). Equation (17) will be called a short run yield equation. At each level of biomass the relation between yield and effort is linear; the slope of the line increases as biomass increases.

In a nonequilibrium fishery the stock size increases as effort decreases, but at the same time an increase in the stock size will increase the amount of effort applied to the fishery because the higher the stock density the greater the yield and revenue per unit of effort. For example, three short run yield curves and an equilibrium yield curve for a hypothetical population are drawn in Fig. 4. Suppose that the stock size is B_2 and the effort is E_0. If at this nonequilibrium point revenue exceeds cost, effort will increase. Biomass also will increase until effort exceeds the equilibrium level. Assume that effort reaches E_1. Initially the biomass will be greater than B_1, but as effort continues at E_1 the biomass will approach B_1 as the stock is fished up. For the fishery to reach an equilibrium at stock size B_1

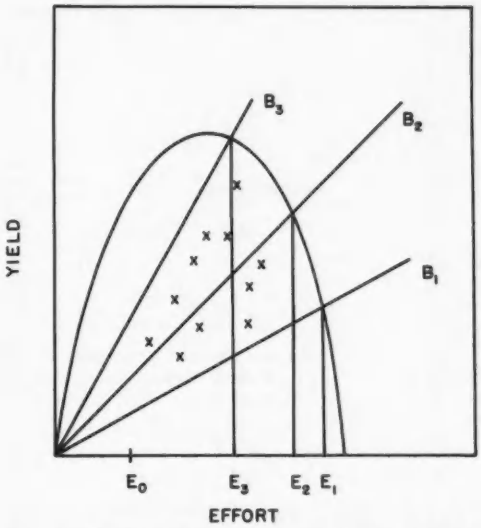

FIG. 4. Hypothetical equilibrium stock production curve and three hypothetical short run nonequilibrium yield curves for an exploited population.

and effort level E_1 costs must equal revenue at this point. If costs are greater than revenue fishermen will begin to reduce their effort before the biomass decreases to B_1; as effort decreases catch decreases and the biomass will begin to increase. The slope of the short run yield curve will gradually increase. At some point revenue will again exceed cost so effort will increase and the cycle begins again. The catch and effort data generated by this process will appear as the X's in Fig. 4.

To describe the whitefish catch and effort data another factor must be considered, namely the large natural fluctuations in abundance. Occasionally environmental conditions are suitable for production of one or more large year-classes. When these year-classes enter the fishery the effort and yield increase; this results in large but fairly regular fluctuations in the observed yield and effort statistics (Fig. 2).

Assessment of the Fisheries

The results of applying the surplus production model to three statistical districts (MM-1, MH-1, MS-4) will be examined in detail. One district was selected from the most productive area of each lake.

The estimates of the parameters are listed in Table 2. The estimates for MM-1 and MS-4 with 4½-inch gillnets as the standard gear, with and without sea lamprey predation, were obtained by

TABLE 2. Estimates of the parameters k, K, and q for the logistic surplus production model.

District	k	K	q
	Gillnets without sea lamprey		
MM-1	0.22	9,160,000	0.17×10^{-5}
MH-1	0.50	1,777,364	0.30×10^{-5}
MS-4	0.38	537,069	0.28×10^{-4}
	Poundnets without sea lamprey		
MM-1	0.34	5,519,320	0.35×10^{-4}
MH-1	0.22	1,777,364	0.31×10^{-4}
MS-4	0.48	242,868	0.27×10^{-3}
	Gillnets with sea lamprey		
MM-1	0.30	8,828,447	0.17×10^{-5}

linearization of equation (2) and application of least squares. All other estimates were obtained using Pella and Tomlinson's (1969) method for obtaining initial guesses. There is close agreement among the estimates considering the variation in the data. The estimates of k vary from 0.22 to 0.50. The estimates of the carrying capacity obtained by least squares are larger than those obtained using Pella and Tomlinson's initial guess vaules. The carrying capacity of district MM-1, where catches have been large, is larger than the carrying capacity of the other districts. Except for district MS-4 the catchability constants are close for each type of gear. With gillnets as the standard gear the catchability constant is 0.17×10^{-5} for district MM-1 and 0.51×10^{-5} for district MH-1. With poundnets as the standard gear the catchability constant is 0.35×10^{-4} for district MM-1 and 0.31×10^{-4} for district MH-1. For both gillnets and poundnets the catchability constant of district MS-4 is lower than for the other districts. Estimates of the parameters for district MM-1 with and without sea lamprey predation are nearly the same.

LAKE MICHIGAN

Stock production curves for district MM-1 in northern Lake Michigan fitted using 4½-inch gillnets and poundnets as the standard gear are shown in Fig. 5 and 6, respectively. As expected, there is considerable scatter of the observations about the equilibrium stock production curve. However, most observations fall to the left of the biomass level at which the MSY would be obtained, which indicates that the stock in this district is overexploited.

Yield can be plotted against either biomass or

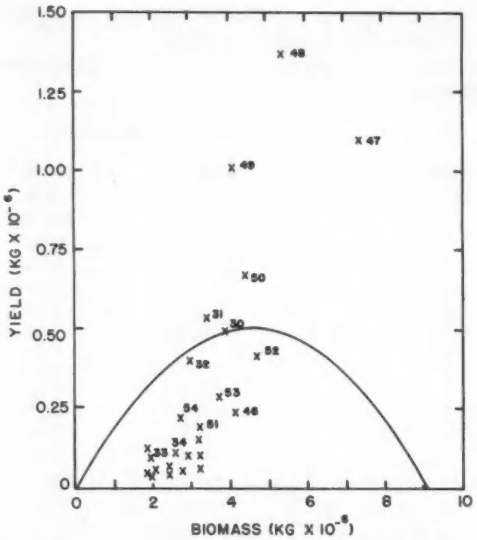

FIG. 5. Equilibrium stock production curve (parabola), and estimated biomass values at observed yields with gillnets as standard gear for district MM-1. The numbers indicate the year for selected observations.

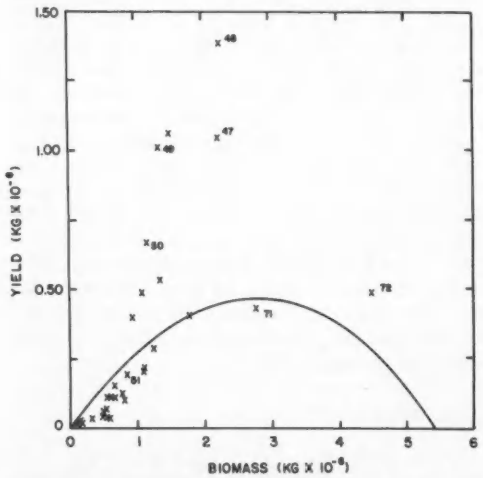

FIG. 6. Equilibrium stock production curve (parabola), and estimated biomass values at observed yields with poundnets as the standard gear for district MM-1. The numbers indicate the year for selected observations.

effort. For biological understanding of a fishery the biomass plot is most useful, and is used in this study. For economic analyses the plot of yield against effort is more useful, and the relation between the equilibrium yield curve and the average short run yield curve is also of interest. The equilibrium relation between yield and effort (equation 16) for district MM-1 with poundnets as the standard gear is drawn in Fig. 7. The regression between yield and effort is also shown in Fig. 7; this regression line is the mean short run yield equation.

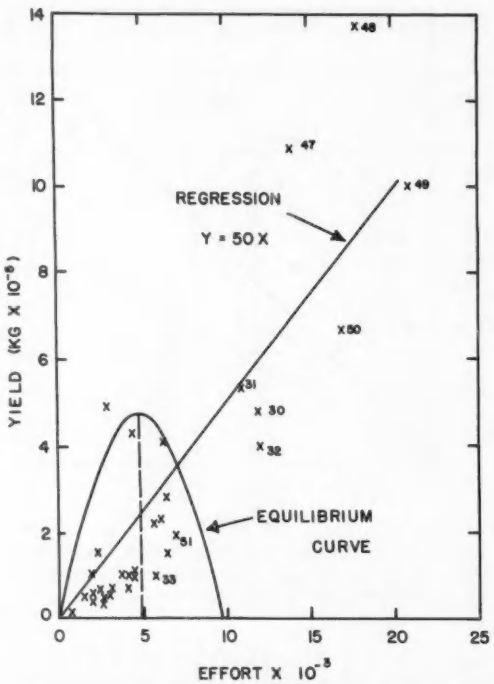

FIG. 7. Estimated equilibrium yield and effort relation (parabola), and regression of yield on effort (solid line). The points are the observed yield and effort values.

As expected, the observations fluctuate considerably about both the equilibrium curve and the mean short run yield curve. The observations at high effort levels occurred during the peak yield years of 1930–32 and 1947–50 (Fig. 2 and 7). These observations resulted from large year-classes entering the fishery and they represent conditions far from equilibrium. The rest of the observations have a pattern similar to that illustrated in Fig. 4 for a nonequilibrium fishery. Many of the points near the origin occurred during the years 1945–60 when the sea lamprey had severely reduced the whitefish population. The mean short run yield curve intersects the equilibrium curve to the right of the maximum which indicates that the stock is overexploited.

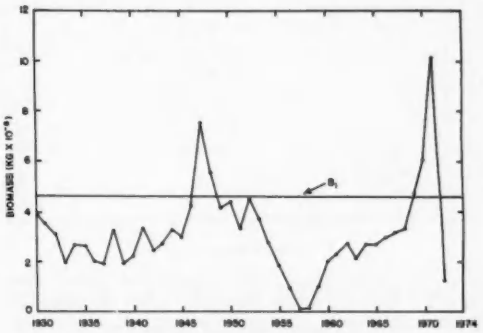

FIG. 8. Estimated biomass levels for district MM-1 with gillnets as standard gear. B_1 is the biomass level at which the MSY would occur.

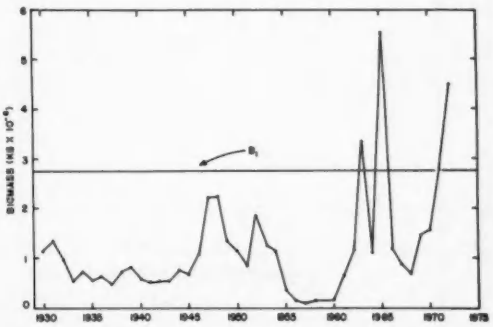

FIG. 9. Estimated biomass levels for district MM-1 with poundnets as standard gear. B_1 is the biomass level at which MSY would occur.

The assumption that the fisheries are not in equilibrium implies that a close relation exists between yield and biomass. Therefore, a good way to compare the results obtained using gillnets and poundnets is to compare biomass estimates for each gear type with the observed yields. The estimates of biomass in district MM-1 made with 4½-inch gillnets and poundnets as the standard gear are shown in Fig. 8 and 9, respectively. In both of these figures B_1 is the biomass that would produce the MSY. Estimates based on both gillnets and poundnets give a similar description of changes in biomass. During the years 1930–45 biomass appeared to be relatively constant, but increased during the 1940s. The decrease in biomass that occurred during the late 1940s and 1950s occurred when the sea lamprey invaded Lake Michigan. The poundnet estimates of biomass indicate that a high biomass level occurred in 1963 and again in 1965. These fluctuations do not occur with the gillnet estimates.

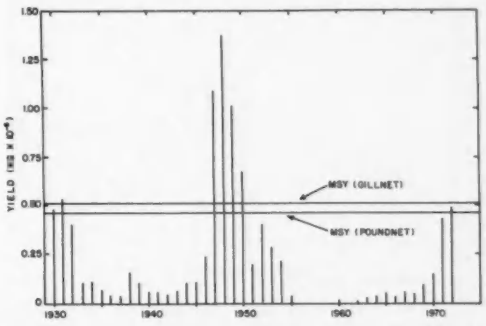

FIG. 10. Observed yields and the MSY with gillnets and poundnets for district MM-1.

Both gillnets and poundnets predicted large biomass levels in the late 1960s, but gillnets indicate a large decrease from 1971 to 1972 that is not indicated by the poundnet estimates.

Comparison of the yield obtained from district MM-1 (Fig. 10) with the biomass estimates indicates that yield and biomass are closely related for both types of gear. Although there are some differences in the biomass estimates, especially after 1960, and although gillnets predict higher biomass levels than poundnets, the two gears gave a similar description of the whitefish fishery in district MM-1, but only in MM-1. In other districts of Lake Michigan, and in Lake Huron and Lake Superior the changes in gillnet efficiency were detected.

Patriarche (1974) applied a different method to estimate the biomass of district MM-1 in 1971 and 1972. The age structure and mean weight of a few commercial yields were determined. From estimates of the yield per age-group and estimates of the exploitation rate, biomass of the exploited stock (age ≥ 3) was estimated as 1.3 million kg in 1971 and 0.9 million kg in 1972. Patriarche's biomass estimates are not too different from the estimates obtained with the surplus production model of approximately 10 and 1 million kg with gillnets and approximately 1.5 and 4.5 million kg with poundnets.

The relative importance of commercial fishing and sea lamprey predation on the decline of the whitefish population in district MM-1 during the 1950s can be determined using equations (3) and (4). Equation (4) was fitted with gillnets as the standard gear by linearization and least squares. Adding the sea lamprey predation term to the surplus production model increased the coefficient of determination from 0.17 to 0.26. The coefficients of determination for the models fitted to districts MH-1 and MS-4 are also about 0.30.

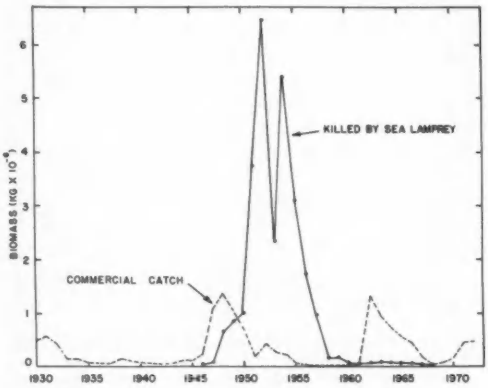

FIG. 11. Catch by the fishery and estimated biomass of whitefish killed by sea lamprey (*Petromyzon marinus*) in district MM-1 of northern Lake Michigan.

The biomass removed by fishermen and the biomass estimated to have been removed by sea lamprey predation are plotted in Fig. 11. The impact of the sea lamprey on the whitefish population was tremendous as compared to the small impact of commercial fishing. These estimates of sea lamprey predation are accurate enough to conclude that although the whitefish stock in this district has been consistently overexploited, the decline in whitefish biomass during the 1950s was caused by the sea lamprey and not by overfishing.

LAKE SUPERIOR

Application of the surplus production model to district MS-4 in Lake Superior gives results that are considerably different from those obtained for district MM-1. The MS-4 stock production curve fitted with 4½-inch gillnets as the standard gear is shown in Fig. 12. For this district observations appear to be available for all stages of the fishery. However, the observations on the right at high biomass levels in Fig. 12 all occurred after 1960. The MS-4 stock production curve with poundnets as the standard gear is shown in Fig. 13. Points far to the right in Fig. 12 appear on the left in Fig. 13; the biomass estimates do not show an increase after 1960 when estimates are based on poundnets. Although the stock production curve fitted using gillnets as the standard gear differs from that using poundnets, neither curve indicates consistent overexploitation.

Biomass estimates for district MS-4 with gillnets and poundnets as the standard gear are shown in Fig. 14, and 15, respectively. After

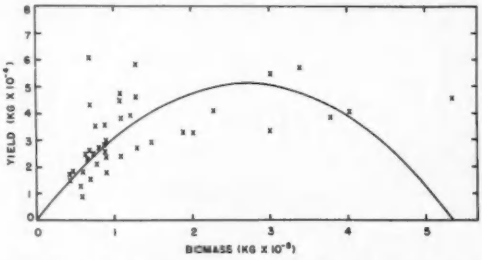

FIG. 12. Equilibrium stock production curve (parabola), and estimated biomass values at observed yields with gillnets as standard gear for district MS-4.

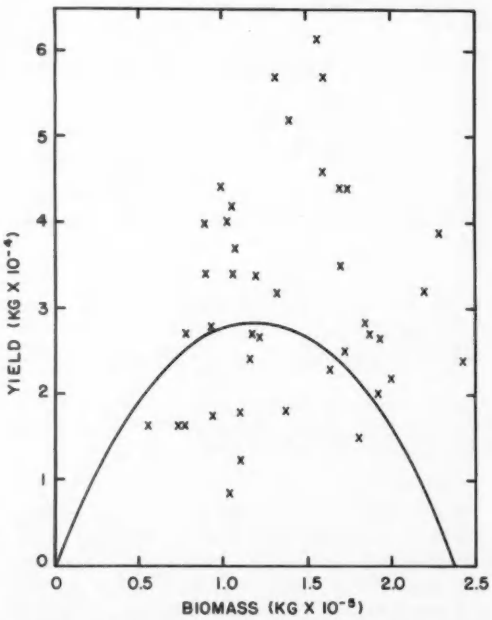

FIG. 13. Equilibrium stock production curve (parabola), and estimated biomass values at observed yields with poundnets as standard gear for district MS-4.

introduction of the monofilament gillnet in about 1960, the estimates of biomass using gillnets begin to increase greatly. Estimates based on poundnets indicate that no increase in biomass occurred after 1960. The biomass estimates based on gillnets and poundnets are not closely related to the observed yields (Fig. 16); however, the pattern of biomass levels predicted by poundnets is closer to the pattern of yield than is the biomass predicted by gillnets. The observed yields do not

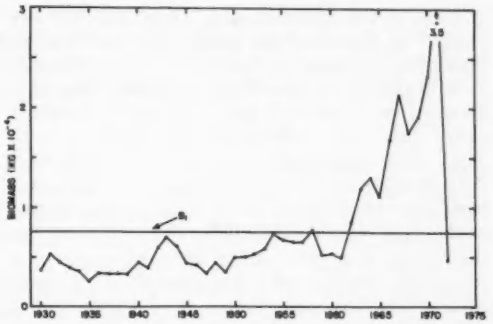

FIG. 14. Estimated biomass levels for district MS-4 with gillnets as standard gear.

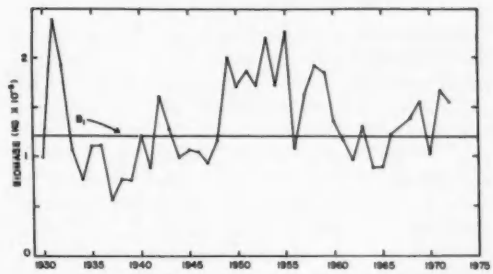

FIG. 15. Estimated biomass levels for district MS-4 with poundnets as standard gear.

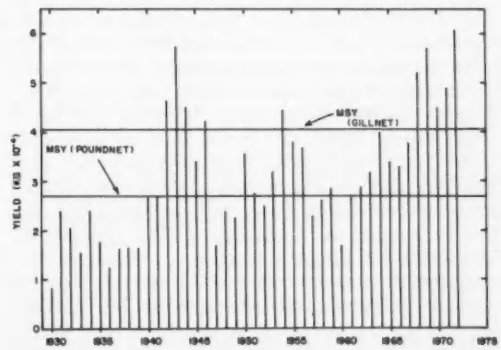

FIG. 16. Observed yields and the MSY with gillnets and poundnets for district MS-4.

indicate that a large increase in biomass occurred after 1960.

The changes in whitefish biomass in northern Lake Michigan and Lake Superior, as predicted by the surplus production model, are similar to the changes described by Smith (1968). In northern Lake Michigan the sea lamprey began to have

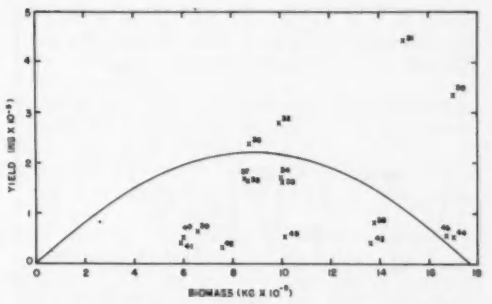

FIG. 17. Equilibrium stock production curve (parabola), and estimated biomass values at observed yields with gillnets as standard gear for district MH-1. The numbers indicate the year for selected observations.

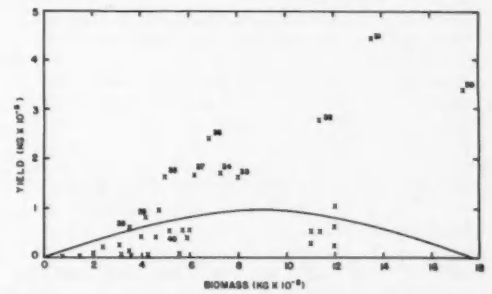

FIG. 18. Equilibrium stock production curve (parabola), and estimated biomass values at observed yields with poundnets as standard gear for district MH-1. The numbers indicate the year for selected observations.

an effect on lake trout in about 1944, and as the population declined fishermen began to transfer effort to whitefish. When the lake trout population reached low levels lamprey began to prey upon whitefish causing a sharp decline in whitefish biomass and yield. After control of the sea lamprey, whitefish recovered rapidly in Lake Michigan. In Lake Superior the whitefish, which live in shallow waters, were protected from sea lamprey predation by the availability of preferred prey species such as lake trout, large chubs (*Leucichthys* spp.), and burbot (*Lota lota*) which live in the deep waters with the sea lamprey. Sea lamprey did not prey heavily upon whitefish in Lake Superior (Smith 1968).

LAKE HURON

In Lake Huron district MH-1 the whitefish stock appears overexploited when poundnets are

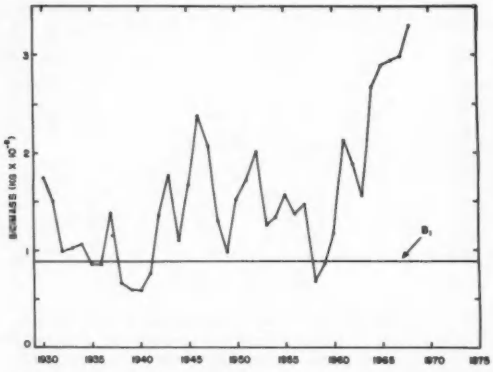

FIG. 19. Estimated biomass levels for district MH-1 with gillnets as standard gear.

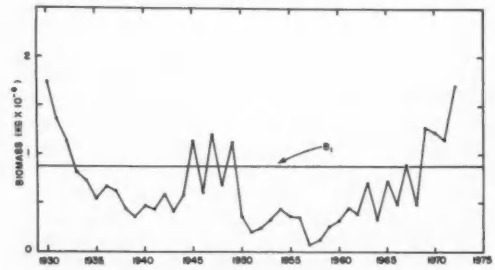

FIG. 20. Estimated biomass levels for district MH-1 with poundnets as standard gear.

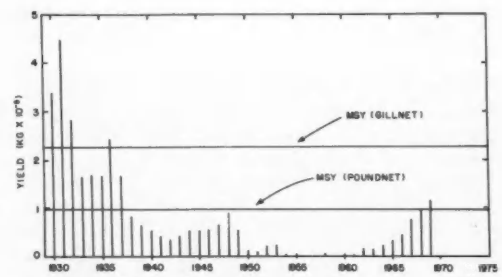

FIG. 21. Observed yields and the MSY with gillnets and poundnets for district MH-1.

used as the standard gear, but appears underutilized using gillnets. The stock production curve with gillnets and poundnets as the standard gear are shown in Fig. 17 and 18, respectively. Only data for 1930–45 are plotted. After 1945 catches from Lake Huron were small.

Poundnets give a better description of the fishery than gillnets. Biomass estimates based on gillnets as the standard gear (Fig. 19) are not related to the observed yields, whereas biomass estimates made using poundnets (Fig. 20) are closely related to the observed yields (Fig. 21). Biomass estimates with poundnets as the standard gear, and yield, both decreased from 1930 to 1943; they increased from 1943 to 1948, and then both decreased to low levels when sea lamprey became abundant in the lake. After control of the sea lamprey, biomass estimates based on poundnets, and yield, again both increased. The poundnet data indicate that the whitefish stock in northern Lake Huron has been overexploited during most of the historical record, and that only in recent years is the population recovering.

Management of the Fisheries

Application of the surplus production model to the whitefish yield and effort data has required many assumptions and approximations, but some suggestions for management of the fisheries appear reasonable. The stocks in most districts are, or have been, overexploited. The level of exploitation of the stocks in some districts of Lake Superior has fluctuated about the optimum level. In the districts where whitefish have been overexploited, yield can be increased by allowing the size of the stock to increase. In districts where overexploitation occurs fishing effort is a function of biomass; an increase in biomass results in an increase in effort when revenue exceeds costs. To interrupt this feedback system which is maintaining low yields it is necessary either to establish yield quotas or limit the amount of fishing effort.

The surplus production model can be applied to each district to determine the MSY and optimum fishing effort. The model should be applied using poundnets as the standard gear. With the available data the parameters of the model cannot be accurately estimated; nevertheless, the models can be applied for management. The application of models for fishery management should be an evolutionary process; the models can be applied now to guide management, and as the fishery approaches the optimum level, the fishery itself can provide data for improving the fit of the model and estimates of the optimum yield. Information on the biological processes of mortality, growth, and recruitment are also necessary to confirm and elaborate upon the surplus production model.

Until economic studies have been completed it is reasonable to manage the fisheries near the MSY, but it does not appear that fishing effort can be easily regulated and the State of Michigan, for example, is investigating catch quotas. For fisheries such as those of the Great Lakes where

large fluctuations occur in the sizes of the stocks the MSY is not useful for establishing catch quotas. One reasonable catch quota, Y_t, for a nonequilibrium fishery is

$$Y_t = q\, E_{opt}\, \hat{B}_t \qquad (18)$$

where E_{opt} is the optimum effort, and $\hat{B}_t$ is the year ahead biomass forecast. The year ahead forecast of biomass can be made using either a surplus production model or an autoregressive model. This method of establishing quotas can, in the long run, give the MSY or MEY depending on the value of E_{opt}. When biomass increases above the optimum level, catch quotas will increase, and when biomass decreases below optimum catch, quotas will decrease. The catch will fluctuate about the optimum, and in the long run the average catch should be the optimum catch.

Applying equation (18) for establishing catch quotas should result in a relatively constant level of effort which should be economically efficient. However, when strong year-classes are passing through the fishery, yield could be increased by increasing effort without harm to the fish stock. When a strong year-class is indicated the catch quota could be increased by adding an appropriate increment such as $\Delta B = \hat{B} - B_1$ to equation (18).

Acknowledgments

Interpretation of the Great Lakes fishery statistics was difficult and I received a great deal of advice and help from S. H. Smith, J. Carr, and H. Buettner of the National Marine Fisheries Service and from M. Patriarche, A. T. Wright, R. L. Eshenroder, and M. Keller of the State of Michigan Department of Natural Resources. S. H. Smith and M. Patriarche read an early version of this manuscript and made many helpful suggestions. H. Buettner provided me with the data. This study was supported by the University of Michigan Sea Grant Program.

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The Number of Genes for Lactate Dehydrogenase in Salmonid Fishes

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BAILEY, G. S., H. TSUYUKI, AND A. C. WILSON. 1976. The number of genes for lactate dehydrogenase in salmonid fishes. J. Fish. Res. Board Can. 33: 760–767.

Rainbow trout (*Salmo gairdneri*) that show polymorphism at the *ldhHα* locus for liver lactate dehydrogenase (LDH) (L-lactate: NAD oxidoreductase, EC 1.1.1.27) have been examined electrophoretically and immunologically. These results show that the *ldhHα* locus is active in two possible allelic forms, $ldhH\alpha^A$ and $ldhH\alpha^B$, in many tissues such as heart, liver, kidney, eye, red blood cell, brain, and pyloric caeca. Quantitative microcomplement fixation tests support the contention that $H\alpha^A$ and $H\alpha^B$ subunits are allelic variants. These results, combined with electrophoretic studies on brook trout (*Salvelinus fontinalis*) polymorphic at the *ldhHβ* locus, lead us to conclude that all isozymes for LDH in salmonid fishes can be accounted for by the activity of four, and possibly five structural loci: *ldhHα*, *ldhHβ*, *ldhC*, *ldhMα*, and possibly *ldhMβ*. These results are in contrast to previous suggestions that the multiple lactate dehydrogenases of trout are encoded by 8 or 10 structural genes per haploid genome.

BAILEY, G. S., H. TSUYUKI, AND A. C. WILSON. 1976. The number of genes for lactate dehydrogenase in salmonid fishes. J. Fish. Res. Board Can. 33: 760–767.

Nous avons examiné par des méthodes électrophorétiques et immunologiques des truites arc-en-ciel (*Salmo gairdneri*) montrant un polymorphisme au locus *ldhHα* pour la lacticodéshydrogénase (LDH) hépatique (L-lactate: NAD oxydoréductase, EC 1.1.1.27). Les résultats démontrent que le locus *ldhHα* agit sous deux formes alléliques possibles, $ldhH\alpha^A$ et $ldhH\alpha^B$, dans plusieurs tissus tels que cœur, foie, rein, œil, globule rouge sanguin, cerveau et cæca pyloriques. Les essais quantitatifs de fixation du micro-complément confirment la prétention que les sous-unités $H\alpha^A$ et $H\alpha^B$ sont des variants alléliques. Ces résultats, combinés à ceux d'études électrophorétiques sur des ombles de fontaine (*Salvelinus fontinalis*) polymorphes au locus *ldhHβ*, nous incitent à conclure que tous les isozymes de la LDH chez les salmonidés peuvent être expliqués par l'activité de quatre, possiblement cinq loci de structure: *ldhHα*, *ldhHβ*, *ldhC*, *ldhMα* et possiblement *ldhMβ*. Ces résultats contredisent des suggestions antérieures à l'effet que les multiples lacticodéshydrogénases des truites sont codées par 8 ou 10 gènes de structure par génôme haploïde.

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SALMONID fishes are well known to possess large numbers of electrophoretically resolvable isozymes for lactate dehydrogenase (L-lactate NAD:oxidoreductase, EC 1.1.1.27); however, information on the total number of structural genes coding for these isozymes is as yet unclear. Detailed genetic analyses (Morrison and Wright 1966; Morrison 1970) combined with biochemical studies (Massaro and Markert 1968; Bailey and Wilson 1968; Wuntch and Goldberg 1970; Lim et al. 1975) have demonstrated the existence of two loci, *ldhHα* and *ldHhβ*, producing a series of five LDH isozymes all of which are structurally homologous to H_4 LDH of higher vertebrates. Studies in salmonids and other fishes have also established the existence of at least one locus producing a C_4 LDH isozyme, expressed largely in retina in salmonids (Morrison and Wright 1966; Whitt 1970; Shacklee et al. 1973; for a

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recent review of the evolution of lactate dehydrogenase isozymes in fishes see Markert et al. 1975). Finally, striated muscle tissues in salmonids are known to possess an electrophoretically distinct set of three to five isozymes whose physical and catalytic properties suggest homology with the M_4 LDH of higher vertebrates (Bailey and Wilson 1968; Wuntch and Goldberg 1970). Although this isozyme series is generally believed to derive from two closely related genes, there is as yet no direct experimental evidence that two structural loci are in fact involved.

This brings the total number of known loci for LDH in salmonid fish to at least four and possibly five. However, the existence in certain tissues of supernumary LDH isozymes of unusual electrophoretic behavior, apparently unaccounted for by the above loci (Williscroft and Tsuyuki 1970; Massaro and Markert 1968), as well as the anomalous behavior of some of these isozymes during in vitro hybridization (Massaro and Markert 1968; Joyce et al. 1973) has led to the suggestion that as many as 8 or 10 structural loci may be necessary to account for all the LDH isozymes observable in extracts of various tissues of salmonid fishes.

We report here an extension of these previous investigations of the multiple forms of LDH in trout in an attempt to determine whether there are additional loci for LDH unique to such tissues as liver, gill, and intestine, or whether all observable LDH isozymes can be accounted for by the four or five loci for which evidence now exists.

Materials and Methods

Rainbow trout (*Salmo gairdneri*) were obtained from Kokanee Creek near Nelson, B.C. Tissues were excised and extracted immediately after the fish were killed. Most tissue extracts were prepared by homogenizing with four volumes of sodium phosphate buffer of pH 7.55, ionic strength 0.05, chilled to 4 C. Intestinal tissue was homogenized with three volumes of buffer; eye tissues were used without further addition of buffer. After centrifugation (15,000 × *g*, 30 min) the clarified supernatant was applied directly to the starch gel.

Starch gel was prepared as a 13% gel in 0.002 M citric acid adjusted to pH 8.0 with tris(hydroxymethyl) aminomethane (Tris) (Clayton and Gee 1969). The electrode solution was 0.04 M citric acid adjusted to pH 8.0 with Tris. Electrophoresis was carried out at 4 C at 200 V as described previously (Tsuyuki et al. 1968) but for 6½ h. The lactate dehydrogenase was visualized as described before (Williscroft and Tsuyuki 1970). Two buffer systems at pH 8.6 and pH 6.0, previously described by Bailey and Wilson (1968), also were employed.

Antisera to $H\alpha_4^n$ liver LDH from rainbow trout were prepared by immunizing two New Zealand White rabbits (F1 and F2) with partially purified liver LDH (Bailey and Wilson 1968) by methods described elsewhere (Lim et al. 1975). Tests for reactivity between antisera and either purified LDH or tissue extracts were carried out by double immunodiffusion and microcomplement fixation (Bailey and Wilson 1968; Champion et al. 1974). Both tests revealed that the antisera contained antibodies to impurities as well as to LDH. This problem was circumvented by using the nitro-blue tetrazolium method (Bailey and Wilson 1968) to identify precipitin bands containing LDH in immunodiffusion tests with tissue extracts and by purifying the LDH from starch gels as described by Karig and Wilson (1971) for use in microcomplement fixation tests.

Purification of $H\beta_4$ and $H\alpha_4^n$ homotetramers and the methods for freeze–thaw molecular hybridization have been reported in detail elsewhere (Lim et al. 1975).

In this communication we introduce a new nomenclature for designation of genetic loci, alleles, and polypeptides of LDH in salmonid fish. This system, which is patterned after those used by geneticists, allows the unambiguous designation of all components in a complicated set of genes which have arisen through several duplication events, and provides for the future naming of as yet undescribed alleles. Each locus is identified by a three-letter code (*ldh* for lactate dehydrogenase loci) followed in the first instance by a capital letter for each polypeptide class in an isozymic system; in this case there are three LDH loci common to all teleosts (Horowitz and Whitt 1972), which we designate *ldhM*, *ldhH*, and *ldhC*. Further recent duplications of loci restricted to the family Salmonidae are designated by addition of Greek letters, e.g. *ldhHα* and *ldhHβ*. Finally, the various alleles for loci which show polymorphism are designated by capitalized Arabic superscripts, e.g. $ldhH\alpha^A$, $ldhH\alpha^B$.

Other systems for designation of LDH isozymes (e.g. A_4–B_4 rather than M_4–H_4) can be easily adapted to our suggested scheme of locus, allele, and isozyme nomenclature, so that *ldhHα* is synonymous with *ldhBα*, *ldhMβ* could be designated *ldhAβ*, the $H\alpha_4^n$ homotetramer would become $B\alpha_4^n$, etc.

Results

Liver

The livers of large numbers of rainbow trout individuals have been tested from wild and hatchery populations in California and New Zealand, and all have been found to exhibit a single major band of LDH activity after electrophoresis in starch gel at pH 6, 8, or 8.6 (Bailey 1969; S. T. Lim and G. S. Bailey unpublished data). However, genetic variation has been shown to occur at the locus coding for this enzyme in certain populations in southern British Columbia

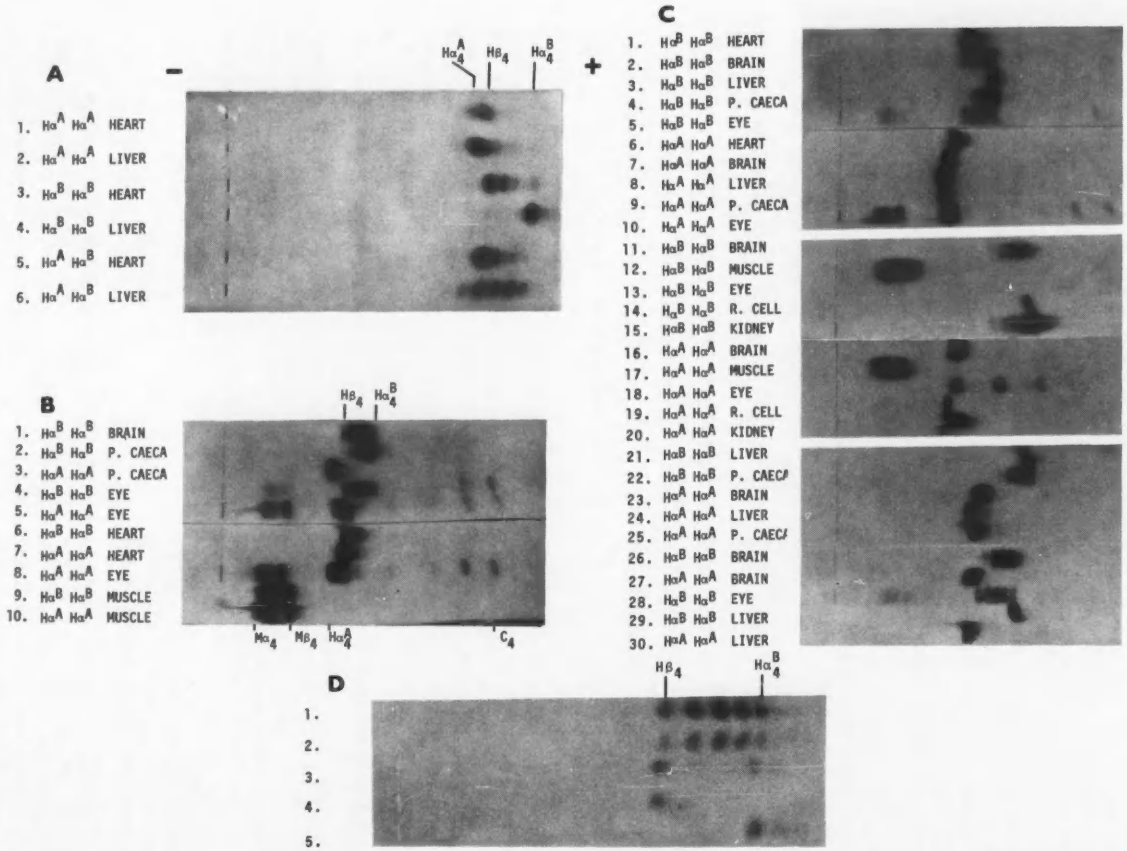

FIG. 1. Starch gel electrophoresis of tissue extracts of rainbow trout (*Salmo gairdneri*) showing variants at the *ldhHα* locus. A, gel was run at pH 8.6 and gels shown in B and C were run in the pH 8.0 buffer system (see Materials and Methods). No reference is made on the figure to the genotypes at the *ldhM*, *ldhHβ*, and *ldhC* loci, which show constant phenotypes in these individuals. Only the *ldhHα* locus shows allelic variation in these individuals, with the alleles being designated $H\alpha^A$ and $H\alpha^B$; D, freeze–thaw molecular hybridization of purified $H\alpha_4^B$ and $H\beta_4$ homotetramers. The isosymes were purified from liver and heart, respectively, and molecular hybridization carried out as described by Lim et al. (1975). Electrophoresis was at pH 7.5 for 18 h at 4 C (Lim et al. 1975). Sample 1) $H\beta_4$ + $H\alpha_4^B$, frozen and thawed; 2) same as (1), except half the quantity; 3) $H\beta_4$ + $H\alpha_4^B$, unfrozen control; 4) $H\beta_4$ alone, frozen and thawed; 5) $H\alpha_4^B$ alone, frozen, and thawed. The $H\alpha_4^B$ preparation has an anodal subband, occasionally observed in preparations from liver, which appears to arise from proteolytic degradation of $H\alpha_4^B$ subunits.

TABLE 1. Immunological comparison of antiserum to purified liver $H\alpha_4^B$ LDH with other purified LDHs from rainbow trout (*Salmo gairdneri*).

Type of LDH		Immunological distance		Inferred no. of amino acid substitutions[b]
Source	Subunit composition	Antiserum 1[a]	Antiserum 2[a]	
Liver	$H\alpha_4^A$	3	1	~1
Heart	$H\beta_4$	9	13	~7

[a]Microcomplement fixation titers of antisera 1 and 2 were 1500 and 2200, respectively.
[b]Estimated from the relation between immunological distance and degree of sequence difference established by Prager et al. (1972) and Champion et al. (1975) for homologous series of lysozymes and azurins.

at an allele frequency ranging from 0 to 0.80 (Huzyk and Tsuyuki 1974). The three phenotypes found in these populations are shown in Fig. 1A. The single liver LDH band found in rainbow trout individuals of the most commonly encountered phenotype (Fig. 1A-4) is replaced in some individuals in the Pacific northwest populations by a single band that moves less rapidly toward the anode (Fig. 1A-2) and in other individuals by a set of five equally spaced bands whose relative proportions are in the ratio of approximately 1:4:6:4:1 (Fig. 1A-6). Williscroft and Tsuyuki (1970) showed that a one-locus, two-allele model accounts for this pattern of variation. Similar conclusions have been reached by Utter and Hodgins (1972) and by Wright et al. (1975) using different rainbow trout populations. We designate the locus *ldhH*α and the alleles *ldhH*α^A and *ldhH*α^B. The subunits encoded by the alleles are designated $H\alpha^A$ and $H\alpha^B$, the corresponding tetrameric enzymes being $H\alpha_4^A$, $H\alpha_3^AH\alpha^B$, $H\alpha_2^AH\alpha_2^B$, $H\alpha^AH\alpha_3^B$, and $H\alpha_4^B$ (Fig. 1A-6).

In support of the proposal that the two types of subunit, $H\alpha^A$ and $H\alpha^B$, are allelic variants, we find that the homotetramers $H\alpha_4^A$ and $H\alpha_4^B$ are nearly identical antigenetically. This is evident from microcomplement fixation experiments with antisera to electrophoretically purified $H\alpha_4^B$ enzyme from rainbow trout liver. The two homotetramers differ by an average of only two immunological distance units, which probably corresponds to a single amino acid substitution per subunit (Table 1).

OTHER TISSUES

The predominant LDH in extracts of several tissues such as red blood cells (Fig. 1C-14, 1C-19), kidney (Fig. 1C-15, 1C-20), and pyloric caeca (Fig. 1B-2, 1B-3) has the same electrophoretic mobility as the liver isozyme from the same individual. Thus individuals having only the variant LDH ($H\alpha_4^A$) in their livers (cf. Fig. 1C-8) have the same variant isozyme in their kidney (Fig. 1C-20), red blood cells (Fig. 1C-19), and pyloric caeca (Fig. 1C-9). It is inferred that the locus coding for liver LDH also codes for the major LDH of kidney, pyloric caeca, and red blood cells.

In brain extracts of those rainbow trout that have only $H\alpha_4^B$ LDH in their livers, electrophoresis reveals a series of five bands of LDH (cf. Fig. 1C-2). One of these bands, the most anodal, corresponds exactly in mobility to that of liver LDH from the same individual (Fig. 1C-3), and we designate the five isozymes as $H\beta_4$, $H\beta_3H\alpha^B$, $H\beta_2H\alpha_2^B$, $H\beta H\alpha_3^B$, and $H\alpha_4^B$. Individuals that produce the electrophoretically altered $H\alpha_4^A$ isozyme in their livers (cf. Fig. 1C-8) also have altered brain phenotypes (Fig. 1C-7). In this case the least anodal isozyme corresponds to the $H\alpha_4^A$ isozyme, with the $H\beta_4$ isozyme remaining unaltered in migration. Brain extracts of heterozygous individuals which produce two types of $H\alpha$ subunit, namely $H\alpha^A$ and $H\alpha^B$, exhibit more complicated electrophoretic patterns owing to the presumed occurrence of 15 possible tetramers. For clarification, these electrophoresis results are summarized in diagrammatic form in Fig. 2, which depicts the major isozyme bands in the three phenotypes arising from allelic variation at the *ldhH*α locus. Owing to similarities in charge under our electrophoretic conditions, not all of the theoretically possible tetramers can be resolved in $H\alpha^AH\alpha^B$ heterozygotes and $H\alpha^AH\alpha^A$ homozygotes. However, the above electrophoretic patterns support the proposal that two separate structural loci, *ldhH*α and *ldhH*β are active in the brain.

The relationship between $H\beta_4$ and $H\alpha_4^B$ was also examined immunologically. The $H\beta_4$ LDH from heart extracts was isolated free of other LDH isozymes electrophoretically and tested for reactivity with antisera to the $H\alpha_4^B$ LDH of liver. Although the two LDH's could not be differen-

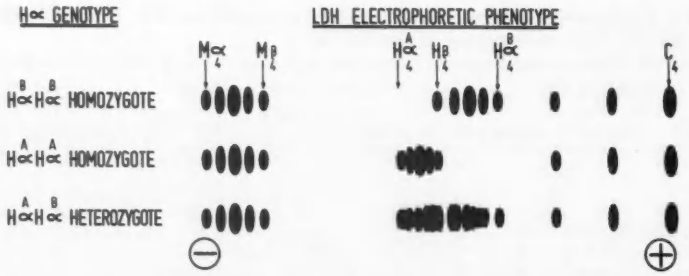

FIG. 2. LDH phenotype for rainbow trout showing genetic variation at only the *ldhHβ* locus. We have exaggerated the difference in mobilities of the $H\beta_4$ and $H\alpha_4^A$ homotetramers to show five distinct $H\beta$–$H\alpha^A$ tetramers; in practice this group of five isozymes cannot be distinctly resolved under our electrophoresis conditions.

tiated by the double immunodiffusion test of Ouchterlony, they were easily distinguishable by the more sensitive microcomplement fixation test. As shown in Table 1, $H\beta_4$ and $H\alpha_4^B$ LDH differ by an average of 11 immunological distance units, which corresponds approximately to seven amino acid substitutions per subunit (Table 1). An analogous observation was made with another salmonid by Lim et al. (1975). This difference is considerably greater than that shown by $H\alpha_4^A$ and $H\alpha_4^B$ (Table 1).

Another tissue in which both the *ldhHα* and *ldhHβ* loci are active is the eye (Fig. 1). Again, individuals homozygous for $H\alpha^B$ exhibit a series of five equally spaced LDH bands in electrophoresis experiments (Fig. 1B-4), while individuals with the $H\alpha^A$ allele exhibit the expected altered electrophoretic pattern (Fig. 1B-5).

In addition to these loci, the *ldhC* locus is active in the eye (Fig. 1). This results in the presence of the $H\beta_4$–$H\alpha_4$ isozyme series, the C_4 homotetramer, and a series of two or three isozymes of intermediate mobility which appear to arise from hybridization of $H\beta$ and C subunits. Allelic variation of the *ldhHα* locus in rainbow trout appears not to affect the mobilities of these intermediate isozymes (Fig. 1B-4, 1B-5) suggesting that the $H\alpha$ polypeptide is not involved in the H–C heterotetramer series. However, variations at the $H\beta$ locus, which occur frequently in brook trout, result in alterations of the mobilities of these intermediate isozymes (Fig. 3) (Bailey 1969; Morrison and Wright 1966). Individuals homozygous for the $H\beta^B$ allele produce the $H\beta^BC_3$ heterotetramer isozyme (Fig. 3-1); heterozygous individuals carrying the $H\beta^A$ and $H\beta^B$ alleles produce the two heterotetramers $H\beta^AC_3$ and $H\beta^BC_3$ in eye extracts (Fig. 3-1, 3-2). No other unique polypeptide or locus is necessary to account for these isozymes.

Finally, the *ldhHα* and *ldhHβ* loci are also active in heart tissue, with the $H\beta$ polypeptides predominating over $H\alpha$ (Fig. 1B-6). Unless blood and other tissue types are carefully excluded during tissue homogenization, the pattern of isozymes in "heart" extracts can appear to be nonbinomial; this is often unavoidable with frozen samples (Fig. 1A-3). However, the $H\beta_4$ isozyme partially purified from heart (sp act 300 U/mg) readily undergoes freeze–thaw molecular hybridization with the $H\alpha_4^B$ homotetramer from liver, generating apparently binomial patterns (Fig. 1D).

Discussion

The major lactate dehydrogenase isozyme in the liver of rainbow, when partially purified from preparative starch gels, appears not to undergo molecular hybridization in vitro with the $H\beta_4$ homotetramer of heart tissue, nor does an enzyme of similar mobility from pyloric caeca (Massaro and Markert 1968; Joyce et al. 1973). This behavior, coupled with the unusual electrophoretic mobilities of some of the isozymes, suggested that the intestinal enzymes and possibly the liver enzyme might be the products of genes that are not identical in structure to the locus active in heart and brain tissue.

The results presented here demonstrate that in fact a single structural gene, *ldhHα*, produces the $H\alpha$ subunit in heart, liver, brain, eye, kidney, red cell, liver, and intestine. The possibility was previously noted (Holmes and Markert 1969; Massaro 1973) that the intestinal series of isozymes might arise from postsynthetic modification of the polypeptide which we term $H\alpha$; our results confirm this suggestion. Furthermore, the previous reports of failure of heart $H\beta_4$ and liver $H\alpha_4^B$ to undergo in vitro molecular hybridization appear to be due to the presence of interfering sub-

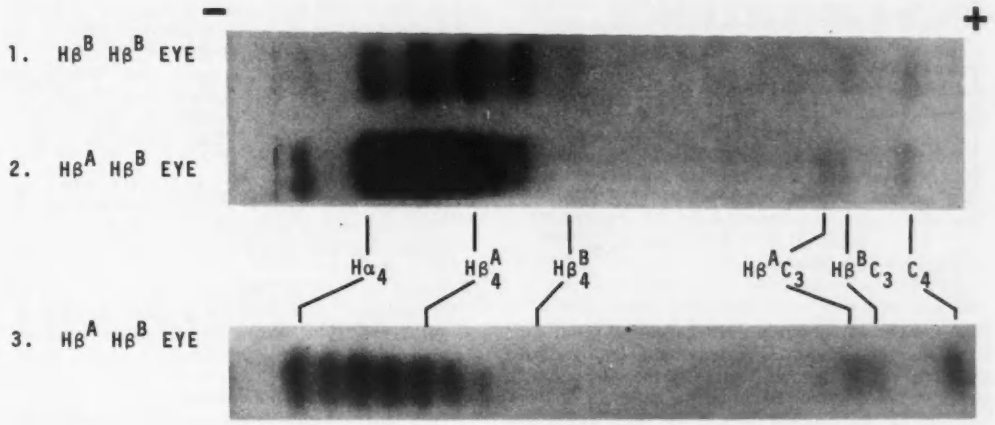

FIG. 3. Electrophoresis of eye extracts of brook trout (*Salvelinus fontinalis*) showing polymorphism at the *ldhHβ* locus. Electrophoresis was at pH 6 for 18 h at 4 C. The relative mobilities of the $H\alpha_4$ and $H\beta_4$ isozymes in brook trout are the reverse of those in rainbow trout (Bailey 1969). 1, an individual homozygous for $H\beta^B$; 2, a heterozygous individual carrying the $H\beta^A$ and $H\beta^B$ alleles; 3, the same individual as 2, except electrophoresis was carried out for 24 h for improved resolution of the $H\beta^AC_3$ and $H\beta^BC_3$ heterotetramers.

stances in the enzyme preparations; the results presented here show that these two isozymes hybridize in an apparently unrestricted fashion both in vivo and in vitro.

Two unique genes have been also proposed to account for the series of LDH isozymes unique to eye and neural tissues in salmonids, as would be expected from the tetraploid origins of these fish (Massaro and Markert 1968). However, the evidence presented here, in combination with earlier work (Morrison and Wright 1966; Holmes and Markert 1969; Morrison 1970), demonstrates that the LDH isozymes unique to these tissues can be totally accounted for through the hybridization of $H\beta$ subunits and a single eye LDH polypeptide. It might, of course, be possible that the C_4 isozyme represents the electrophoretically identical products of two (or more) structural loci; such a situation has been documented for duplicated genes for cytoplasmic malate dehydrogenase in salmon and trout (Bailey et al. 1970). Alternatively, one of the two *ldhC* loci expected to have arisen through tetraploidy may have subsequently been eliminated during evolution. In support of the latter proposal, a population of Arctic char (*Salvelinus alpinus*) has recently been discovered which shows electrophoretic variants of the C polypeptide; the mobilities and distribution of isozymes in the three observable phenotypes suggest that only one locus codes for C polypeptides in this species (J. W. Clayton and D. M. Blouw personal communication).

Finally, the series of LDH isozymes in rainbow trout gill extracts which were tentatively suggested to be the product of two further unique LDH loci (Williscroft and Tsuyuki 1970) have been found to have thermal stability, immunological properties, and electrophoretic mobilities characteristic of isozymes in the $M\alpha_4$–$M\beta_4$ series (Lim et al. 1975; S. T. Lim and G. S. Bailey unpublished data).

Any proposal for the existence of a new gene product should subsequently be substantiated by application of the following criteria: 1) thorough biochemical characterization of the polypeptide product of the putative new locus is essential; if the product was originally identified by unique electrophoretic mobility, the molecular basis of that mobility must be established; 2) detailed genetic studies must be carried out; if the new polypeptide is proposed to be genetic in origin, its pattern of inheritance must be examined. These criteria have not yet been applied to the $M\alpha_4$–$M\beta_4$ LDH series in salmonid fish, nor were they met for the proposed five additional genes for LDH in trout whose existences are questioned in this communication.

We therefore conclude that there is to date substantial evidence for the existence of the following loci for the production of LDH in salmonid fish: *ldhHα*, *ldhHβ*, *ldhC*, and at least one locus for M-type polypeptides, namely *ldhM*. We are currently examining the primary sequence of the $M\alpha_4$–$M\beta_4$ isozyme series in chinook salmon (*Oncorhynchus tshawytscha*) to determine whether the electrophoretically resolvable $M\alpha$ and

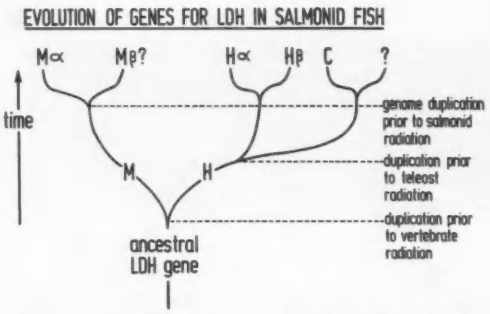

FIG. 4. The evolutionary development of genes for LDH in salmonid fish. The horizontal and vertical axes are not drawn to scale. In the absence of definitive proof we consider that the existence of two loci for M polypeptides is still an open question. Since available evidence implies the existence in living salmonids of only one *ldhC* locus, then one of the pair of C loci arising from tetraploidy appears to have been subsequently eliminated.

Mβ polypeptides are the products of two genes, $ldhM_\alpha$ and $ldhM_\beta$, or whether the series results from postsynthetic modification of the product of a single *ldhM* locus. The M_α and Mβ polypeptides cannot be the result of allelic variation about a single *ldhM* locus, since every individual examined to date has both M_α and Mβ, but the possibility of epigenetic modification of a single gene product cannot yet be ruled out.

Our concept of the evolutionary development of the genes for LDH production is salmonid fish is summarized in Fig. 4. From the results of quantitative microcomplement fixation experiments, we have estimated the time of genome duplication in salmonids which gave rise to $ldhH_\alpha$ and $ldhH_\beta$ at approximately 80–100 million yr ago (Lim et al. 1975); the duplication of the ancestral *ldhH* locus which gave rise to *ldhC* occurred approximately 300 million yr earlier (D. L. Willcox and G. S. Bailey unpublished data).

Acknowledgments

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Note: After this manuscript was submitted, an article was published (Wright et al. 1975) with similar evidence on the nongenetic origin of the intestinal series of isozymes in rainbow trout.

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Food and Food Selectivity of the Black Bullhead, *Ictalurus melas*, in Lake Poinsett, South Dakota

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REPSYS, A. J., R. L. APPLEGATE, AND D. C. HALES. 1976. Food and food selectivity of the black bullhead, *Ictalurus melas*, in Lake Poinsett, South Dakota. J. Fish. Res. Board Can. 33: 768–775.

Food habits and food selectivity of the black bullhead (*Ictalurus melas*) in Lake Poinsett, South Dakota, were studied from March to November 1970. The black bullhead preyed upon a wide range of food sources in the limnetic, littoral, and benthic zones and seasonal differences in diet occurred in response to availability of prey species. The total food volume in stomachs of 558 black bullheads (143–304 mm total length) comprised about 32% planktonic crustaceans, 25% chironomids, 27% fish, 9% crayfish, 2% filamentous algae, and 5% miscellaneous organisms. Planktonic crustaceans made up 94.4% and chironomids made up 5.6% of the volume of food ingested by 220 young-of-the-year black bullheads. Black bullheads were most selective for the largest cladocerans available, *Leptodora kindtii, Daphnia pulex*, and *D. galeata mendotae*, and for *Chironomus* spp. pupae. Black bullheads were least selective for *Diaphanosoma leuchtenbergianum*, copepods, and chironomid larvae. The black bullhead in Lake Poinsett was primarily a selective planktivore throughout its life history.

REPSYS, A. J., R. L. APPLEGATE, AND D. C. HALES. 1976. Food and food selectivity of the black bullhead, *Ictalurus melas*, in Lake Poinsett, South Dakota. J. Fish. Res. Board Can. 33: 768–775.

Nous avons étudié régime alimentaire et le choix de la nourriture de la barbotte noire (*Ictalurus melas*) dans le lac Poinsett, Dakota-Sud, de mars à novembre 1970. La barbotte noire tire sa nourriture d'une grande variété de sources dans les zones limnétique, littorale et benthique, et son régime alimentaire montre des différences saisonnières selon la disponibilité des espèces proies. Le volume total de nourriture dans les estomacs de 558 barbottes noires (143–304 mm de longueur totale) comprend environ 32% de crustacés planctoniques, 25% de chironomides, 27% de poissons, 9% d'écrevisses, 2% d'algues filamenteuses et 5% d'organismes divers. Les crustacés planctoniques constituent jusqu'à 94.4% et les chironomides jusqu'à 5.6% du volume de nourriture absorbée par 220 jeunes barbottes noires de l'année. Les barbottes noires montrent une préférence pour les plus grands cladocères disponibles, *Leptodora kindtii, Daphnia pulex* et *D. galeata mendotae*, et pour les pupes de *Chironomus* spp. Elles choisissent le moins souvent *Diaphanosoma leuchtenbergianum*, des copépodes et des larves de chironomides. Durant tout son cycle vital, la barbotte noire du lac Poinsett est avant tout un poisson planctonovore sélectif.

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SIZE-SPECIFIC predation by fishes upon zooplankton populations may eliminate large crustacean species or reduce them to insignificant densities. Ecosystem alteration resulting from fish predation has been described by Hrbáček et al. (1961), Brooks and Dodson (1965), Galbraith (1967), and Hurlbert et al. (1972). These studies have demonstrated that feeding behavior and population dynamics of fishes near the top of the food chain have profound effects on the species composition and abundance of zooplankton. Knowledge of food selectivity by fishes is essential for evaluating competition between species and for understanding predator–prey relationships.

The black bullhead, *Ictalurus melas*, is a predominant fish species of the South Dakota Glacial Lake District. Congdon (1968) estimated that in 1967 the black bullhead numerically composed

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about 40% of the total fish population in Lake Poinsett, South Dakota. Other major species are bigmouth buffalo, *Ictiobus cyprinellus*, white crappie, *Pomoxis annularis*, black crappie, *P. nigromaculatus*, fathead minnow, *Pimephales promelas*, and spottail shiner, *Notropis hudsonius*. Zooplankton cycles in Lake Poinsett indicated intensive predation by fishes on large crustacean species throughout the summer of 1968 (Applegate et al. 1973). The zooplankton composition during the spring, April–May, was primarily *Daphnia pulex*, *Cyclops bicuspidatus thomasi*, and *C. vernalis*. The simplified zooplankton associations in the spring were followed by successions as described by Hrbáček et al. (1961) for lakes exhibiting piscine predation. *Daphnia pulex* densities decreased from an estimated density of 90/liter in May to estimated mean monthly densities of 1.2–4.3/liter during the remainder of the year. Population data for *D. pulex* indicated a sevenfold increase in birth rate and a fourfold increase in mortality rate when the population was declining. Invertebrate predators such as *Leptodora kindtii* and *Chaoborus* were not present during the population decline. The species composition during the summer and fall was primarily that of small species: *Bosmina longirostris*, *Chydorus sphaericus*, *Diaphanosoma leuchtenbergianum*, *Daphnia galeata mendotae*, and *Diaptomus* spp.

Food selectivity studies of bigmouth buffalo, a major planktivore in Lake Poinsett, indicated that this species consumes most planktonic organisms in about the same proportion as they are available and probably is not primarily responsible for the alteration of invertebrate species cycles (Starostka and Applegate 1970). Objectives of the present study were to determine if the black bullhead is selective for particular invertebrate species and capable of altering their population structure in Lake Poinsett.

Study Area

Lake Poinsett, the largest natural lake in South Dakota, is in the east central part of the state. The lake has a surface area of 3,682 ha, maximum depth 5.5 m, and mean depth 1.8 m. Thermal stratification never develops in the summer and ice cover is present from November through March. Shifting sand, gravel, and rubble substrata in shoreline areas intergrade with a clay–muck substrate in the lake basin. Higher aquatic vegetation is lacking. Lake Poinsett is a hard-water lake with high dissolved solid and organic matter content. The lake is characterized by a late winter and early spring maximum of unicellular phytoplankton under the ice, and summer blooms of blue-green algae. Unicellular algae provide a food base for the rapid development of planktonic herbivore populations in the spring (Applegate et al. 1973).

Methods

Black bullhead subadults and adults ranging from 143 to 304 mm total length were captured with seines, gillnets, trapnets, and otter trawls from March to November 1970 at 2-wk intervals. Black bullhead young-of-the-year, 37–87 mm in total length, were captured with dipnets and beach seines from August 4 to September 14, 1970 at weekly intervals. All samples except one sample of subadults and adults obtained in June were captured during daylight hours. No attempt was made to differentiate between the feeding habits of fish captured by the various means. Black bullheads were preserved in 10% formalin immediately after capture. Stomach contents were identified and counted, and volumetric measures were made by water displacement in calibrated centrifuge tubes. Cladocerans and copepods were identified to species and insects to genus.

Zooplankton samples were taken from June to November 1970. Duplicate zooplankton samples were taken, at the same time and from the same areas as black bullhead samples, by oblique tows with a metered Miller Sampler (Miller 1961) equipped with a no. 10 mesh net. Oblique tow samples were representative of all depth strata, and the volume of water sampled was 304–1837 liters. Plankton samples were concentrated to fixed volumes (60–150 ml) and three 1-ml subsamples were counted in a circular counting chamber at 30×. All *Leptodora kindtii* in net samples were counted. Measurements of crustaceans were made to determine the relative size of species present. Size measurements of *L. kindtii* were made from the anterior edge of the head to the posterior edge of the third abdominal segment. Measurements of other cladocerans were made from the anterior edge of the head to the posterior edge of the carapace or base of the tail spine. Copepods were measured from the anterior edge of the head to the posterior edge of the caudal ramus. Density of benthic insects was obtained from a study of benthic fauna (Smith and Hales 1972) conducted concurrently with this study. Benthic organisms were sampled with an Eckman dredge (202.5 cm^2) and an Orange Peel dredge (171.1 cm^2). Twenty dredge samples were taken, on each sampling date, at about 2-wk intervals (May–September 1970). Dredge samples were washed through a bronze screen (10 mesh/cm with 0.6-mm openings) and macroinvertebrates were removed from the remaining substrate by hand sorting and by the sugar floatation method (Anderson 1959).

The selectivity index of Ivlev (1961) was used to determine if black bullheads selected certain invertebrate species as food. Values obtained by this method range between −1 and +1; negative values indicate selection against an item, or avoidance, and positive values indicate selection for an item. The formula for determining selectivity is

$$E = (r_i - P_i)/(r_i + P_i)$$

TABLE 1. Stomach contents of 558 subadult and adult black bullheads (*Ictalurus melas*) (143–304 mm total length), expressed as percentage number and (in parentheses) percentage volume, Lake Poinsett, South Dakota, 1970. ($T = <0.05$).

Food item	Month								
	March	May	June	July	Aug.	Sept.	Oct.	Nov.	Total
Filamentous algae									
Cladophora sp.	—	—	—	—	—	—	—	—	—
	—	(2.0)	(0.6)	(9.6)	(0.9)	—	—	—	(1.7)
Cladocera									
Daphnia pulex	100.0	97.7	37.3	16.8	23.7	—	—	—	59.6
	(100.0)	(94.3)	(2.2)	(0.5)	(T)	—	—	—	(8.8)
D. galeata mendotae	—	—	—	0.1	5.2	—	—	—	0.1
	—	—	—	(T)	(T)	—	—	—	(T)
Leptodora kindtii	—	—	0.6	28.0	68.5	94.9	57.4	—	21.4
	—	—	(0.3)	(21.6)	(25.3)	(48.1)	(59.1)	—	(23.4)
Diaphanosoma leuchtenbergianum	—	—	—	2.8	15.4	4.6	42.4	100.0	3.5
	—	—	—	(T)	(T)	(T)	(1.0)	(100.0)	(T)
Copepoda	—	0.8	0.3	1.3	2.6	—	—	—	0.7
	—	(T)	(T)	(T)	(T)	—	—	—	(T)
Decapoda									
Orconectes sp.	—	—	T	T	—	—	—	—	T
	—	—	(24.9)	(17.0)	—	—	—	—	(9.0)
Ephemeroptera									
Caenis sp.	—	0.2	0.2	0.2	—	—	—	—	0.1
	—	(0.4)	(T)	(T)	—	—	—	—	(T)
Chironomidae									
Larvae	—	1.3	2.1	31.9	6.6	T	0.2	—	4.1
	—	(2.2)	(1.2)	(12.8)	(3.1)	(T)	(0.5)	—	(2.7)
Pupae	—	—	56.7	18.7	1.2	0.4	—	—	9.7
	—	—	(62.8)	(38.1)	(1.1)	(0.6)	—	—	(22.4)
Fish[a]	—	—	T	—	0.1	T	T	—	T
	—	—	(6.5)	—	(48.4)	(51.3)	(39.4)	—	(27.2)
Miscellaneous[b]	—	0.1	2.7	0.2	T	—	—	—	0.4
	—	(0.9)	(1.3)	(0.5)	(20.9)	—	—	—	(4.5)
No. bullheads examined	10	61	116	118	113	51	76	13	558
No. bullheads with food	3	43	76	86	72	19	32	3	334
Avg food volume (ml) per stomach[c]	0.1	0.8	1.3	0.6	1.5	3.0	3.0	0.3	1.0
Avg food number per stomach[c]	112.8	685.4	97.2	56.3	46.1	204.0	36.8	12.8	142.4

[a]White crappie, black crappie, yellow perch, spottail shiner.
[b]*Hyalella azteca*, *Gammarus lacustris*, Acari, Corixidae, Trichoptera, terrestrial insects, Hirudinea, tiger salamander (*Ambystoma tigrinum*).
[c]Includes empty stomachs.

where r_i is the percentage of an item in the stomachs and P_i is the percentage of the item in the environment.

Results and Discussion

The total food volume of adult and subadult black bullheads comprised about 32% planktonic crustaceans, 25% chironomid larvae and pupae, 27% fish, 9% crayfish, 2% filamentous algae, and 5% miscellaneous food organisms (Table 1). *Leptodora kindtii* and *Daphnia pulex* were the most important zooplankters in the diet. *Daphnia pulex* made up 100% of the food volume in March and 94% of the food volume in May. *Leptodora kindtii* became a major food item in July when it constituted 22% of the monthly food volume. Consumption of *L. kindtii* increased in August, September, and October to 25, 48, and 59%, respectively. Highest predation on *L. kindtii* in terms of average number of organisms per stomach (194) occurred in September (Table 2). The lower digestive tracts of black bullheads examined during September contained the remains of an average of 1540 *L. kindtii* per fish.

TABLE 2. Mean number of crustaceans per liter in lake samples, (in parentheses) mean number per stomach in subadult and adult black bullheads, and selectivity indices for crustaceans by black bullheads from Lake Poinsett, South Dakota, 1970. (T = <0.05)

Organism, length, and avg vol	June			July			Aug.		Sept.	Oct.			Nov.	
	4	18	30	8	17	31	18	29	14	4	16	30	2	6
Daphnia pulex (<2.4 mm, 0.68 μliters)	8.3 (38.6) +0.66	25.5 (10.7) +0.24	16.3 (48.8) +0.54	10.2 (41.4) +0.63	3.2 (11.6) +0.80	2.2 (1.2) +0.90	1.0 (0.4) +0.53	0.1 −1.0						
D. galeata mendotae (<1.8 mm, 0.19 μliters)	T −1.0	T −1.0			0.2 (0.1) +0.20	1.6 (0.1) +0.36	4.0 (1.2) +0.41	1.5 (3.9) +0.14						
Leptodora kindtii (<12.0 mm, 4.22 μliters)			0.1 (2.4) +0.92	0.1 (24.2) +0.99	0.2 (25.0) +0.99	0.2 (1.9) +0.99	0.3 (7.6) +0.99	0.2 (60.6) +0.99	0.1 (193.5) +0.99	0.1 (40.0) +0.99	T (5.2) +0.99	T (0.4) +0.99	T −1.0	T −1.0
Diaphanosoma leuchtenbergianum (<1.2 mm, 0.14 μliters)	T −1.0		T −1.0		1.3 (1.4) +0.47	22.4 (2.0) +0.50	30.5 (5.7) +0.19	8.8 (8.7) −0.33	10.2 (9.5) −0.75	12.2 (6.8) −0.45	6.3 (30.3) +0.62	2.4 (0.3) +0.57	2.7 −1.0	1.7 −1.0
Diaptomus spp. *D. clavipes* (<2.5 mm, 0.50 μliters)	21.9 (0.2)	10.1	19.0 (0.4)	24.4 (1.4)	30.8 (0.8)	43.5 (0.1)	35.2 (0.4)	6.2 (T)	8.0	11.1	16.3	12.8	8.6	11.7
D. siciloides (<1.3 mm, 0.10 μliters)	−0.98	−1.0	−0.95	−0.88	−0.88	−0.85	−0.84	−0.99	−1.0	−1.0	−1.0	−1.0	−1.0	−1.0
Cyclops vernalis (<1.8 mm, 0.04 μliters)	10.1 (0.1) −0.98	6.1 −1.0	23.3 (0.3) −0.97	38.6 −1.0	63.9 (0.3) −0.98	112.0 (0.1) −0.94	48.7 −1.0	21.7 (2.2) −0.90	13.1 −1.0	8.5 −1.0	8.9 −1.0	5.5 −1.0	6.2 −1.0	4.1 −1.0

TABLE 3. Mean number of benthic organisms per square meter,[a] (in parentheses) mean number per stomach in subadult and adult black bullheads, and selectivity indices for benthic organisms by black bullheads from Lake Poinsett, South Dakota, 1970. (T = <0.05)

Organism	May 1	June 4	June 18	July 17	July 31	Aug. 18	Aug. 29	Sept. 14
Chironomus spp.	58	295	1472	1765	16	257	304	525
larvae	(0.5)	(T)	(4.1)	(7.8)	(0.1)	(1.7)	(0.9)	(T)
	−0.27	−0.99	−0.01	−0.64	+0.56	−0.36	+0.18	−0.91
Other Chironominae	136	1023	477		601			
larvae	(4.1)	(1.3)	(T)		(0.3)			
	+0.34	−0.94	−0.94		−0.55			
Procladius sp.	407	762	315	512	99	118	197	
larvae	(3.9)	(0.1)	(0.1)	(20.5)	(0.2)	(2.9)	(0.1)	
	−0.22	−0.99	−0.79	+0.33	+0.07	+0.27	−0.60	
Chironomidae		163	20	37	140	25	4	6
pupae		(97.5)	(2.2)	(18.8)	(0.6)	(1.0)	(T)	(0.8)
		+0.87	+0.95	+0.92	+0.42	+0.48	+0.66	+0.98
Mayfly (*Caenis* sp.)	62	47			8			
nymphs	(1.4)	(0.3)			(0.3)			
	+0.20	−0.75			+0.91			

[a]From Smith and Hales 1972.

Chironomid pupae were more frequently eaten than larvae; they made up 22% of the yearly food volume, whereas the larvae contributed only about 3%. Chironomid pupae ingested were 99% *Chironomus* spp., the largest sized genus of Chironomidae in Lake Poinsett. Extremely few pupae of the smaller *Procladius* sp. were found in bullhead stomachs. Of the annual total number of chironomid larvae in stomachs, 48% were *Procladius* sp., 35% *Chironomus* spp., 13% *Cryptochironomus* sp. and *Parachironomus* sp., and 4% other genera.

White crappies, black crappies, and yellow perch (*Perca flavescens*), 53–145 mm total length, made up 95% of the fish component of the annual diet. Fish represented 48% of the monthly food volume in August and 51% in September. Crayfish (*Orconectes* sp.) and the filamentous green algae, *Cladophora* sp., were seasonally important food categories in June and July. Crayfish contributed 25% to the monthly food volume in June. Ingestion of *Cladophora* increased to a yearly maximum of nearly 10% of the food volume in July. The average volume and number of food organisms per stomach declined in November when water temperature decreased from 15 to 5 C.

The relative abundance of crustacean species in Lake Poinsett and in stomachs of black bullheads, 143–304 mm total length, indicated that large cladocerans, when they were available, were fed upon in higher proportions than small cladocerans and copepods (Table 2). Average selectivity indices for the cladocerans, *L. kindtii* (<12.0 mm, average vol 4.22 μliters), *D. pulex* (<2.4 mm, 0.68 μliters), *D. galeata mendotae* (<1.8 mm, 0.19 μliters), and *Diaphanosoma leuchtenbergianum* (<1.2 mm, 0.14 μliters) were +0.98, +0.66, +0.28, and +0.10, respectively. Average selectivity indices for *Diaptomus* and *Cyclops* were −0.95 and −0.98, respectively.

The relative abundance of benthic insects in Lake Poinsett and in black bullhead stomachs and the selectivity indices for benthic insects indicate that chironomid pupae and mayfly nymphs were selected foods from the benthic fauna (Table 3). Chironomid pupae (99% *Chironomus* spp.) were positively selected on each sampling date (mean index +0.75). highest consumption of chironomid pupae was observed on June 4 and July 17 when major emergences of the winter and summer generations of *Chironomous* spp. were in progress. Nymphs of the small mayfly, *Caenis* sp., were generally positively selected when they were available (mean index +0.12). Chironomid larvae had negative average selectivity indices. Mean selectivity indices for the most frequently ingested species, *Procladius* sp. and *Chironomus* spp., were −0.27 and −0.31, respectively. Other chironomid larvae, mainly *Cryptochironomus* sp., had a mean selectivity index of −0.52. *Parachironomus* sp. inhabits *Cladophora* of the littoral zone of Lake Poinsett and was not included in selectivity calculations because of its absence in Ekman dredge samples.

Subadult and adult black bullheads did not appear to efficiently use the population of chironomid larvae in Lake Poinsett. Negative selectivity indices for chironomid larvae indicate that larvae inhabiting the benthic substrata may

TABLE 4. Stomach contents of 220 young-of-the-year black bullheads from Lake Poinsett, South Dakota, August 4 to September 14, 1970. (T = <0.05 ml)

Organism	Total no.	Total vol (*ml*)	Percent of total no.	Percent of total vol
Zooplankton				
Cladocera				
Diaphanosoma leuchtenbergianum	84,336	11.8	63.5	35.3
Leptodora kindtii	4,558	17.1	3.4	51.2
Daphnia galeata mendotae	2,664	0.5	2.0	1.5
D. pulex	1,113	0.3	0.8	1.0
Kurzia latissima	6,146	0.1	4.6	0.2
Pleuroxus trigonellus	232	T	0.2	T
P. procurvus	12	T	T	T
Chydorus sphaericus	64	T	T	T
Scapholeberis kingi	15	T	T	T
Copepoda				
Cyclops vernalis	26,867	1.1	20.2	3.2
Diaptomus siciloides	5,070	0.5	3.8	1.5
D. clavipes	292	0.2	0.2	0.4
Benthos				
Chironomid larvae	1,138	1.5	0.9	4.5
Chironomid pupae	287	0.4	0.2	1.1
Corixidae	13	T	T	T
Acari	13	T	T	T
Ostracoda	32	T	T	T

not have been highly vulnerable to black bullhead predation. On the other hand, *Chironomus* spp. pupae, which remain sessile in and on bottom sediments until emergence, were apparently vulnerable to black bullhead predation and were highly selected prey. Although prepupal *Procladius* sp. larvae were present in stomach contents, only one fully developed pupa was recovered. The pupae of *Procladius* sp., like those of many Tanypodinae, have well-developed anal fins and are strong swimmers. Consequently the pupae of *Procladius* sp., unlike the generally sessile pupae of *Chironomus* spp., were apparently capable of evading foraging black bullheads, particularly when more vulnerable prey was available.

Planktonic crustaceans made up 94.4% and chironomids 5.6% of the volume of food ingested by 220 young-of-the-year black bullheads examined. The bulk of the food consisted of the cladocerans, *L. kindtii* and *D. leuchtenbergianum* (Table 4). The relative abundance of planktonic crustaceans and selectivity indices indicate that the densty of preferred food was low and that young black bullheads were selective predators (Table 5).

Because black bullheads are relatively late spawners, the young do not hatch until after the crustacean populations in the lake have been subjected to intensive mortality by the predation of a number of other fishes: fathead minnow, spottail shiner, bigmouth buffalo, carp, white crappie, black crappie, adult black bullheads, and 10 others. Populations of *Daphnia pulex* and *D. g. mendotae* were severely reduced by the time young-of-the-year black bullheads appeared. *Daphnia* density was <3/liter, and the average number per stomach was 35.5 and 9.7 on August 4 and 10, respectively. The *D. pulex* population collapsed by August 31 and *D. g. mendotae* population had declined to an estimated 0.07/liter by September 14. Selectivity indices indicate *Daphnia* was a highly selected food item. *Leptodora kindtii*, the large predatory cladoceran, was a most preferred food item of young-of-the-year black bullheads. Although the density of *L. kindtii* never exceeded 0.3/liter, this crustacean contributed 51.2% of the food volume recovered from young-of-the-year black bullheads. The high selectivity indices for *L. kindtii* by all age-groups of black bullheads demonstrate that this species seeks out this large transparent cladoceran. A similar type of selectivity was observed in Beaver Reservoir, Arkansas, where the transparent phantom midge, *Chaoborus*, made up 98% of the volume of food of black bullheads in the limnetic zone (Applegate and Mullan 1967).

Diaphanosoma leuchtenbergianum was the

TABLE 5. Crustacean density (No./liter), in parentheses average number per stomach of young-of-the-year black bullheads, and selectivity indices for crustaceans by black bullheads from Lake Poinsett, South Dakota, 1970.

Species	Aug. 4	Aug. 10	Aug. 31	Sept. 14
Daphnia pulex	1.3	0.6		
	(20.2)	(2.0)		
	+0.44	+0.73		
D. galeata mendotae	0.6	1.8	0.8	0.1
	(15.3)	(7.7)	(28.3)	
	+0.66	+0.76	+0.36	−1.0
Leptodora kindtii	0.1	0.2	0.1	0.1
	(1.3)	(24.5)	(56.3)	(4.1)
	+0.33	+0.99	+0.94	+0.25
Diaphanosoma leuchtenbergianum	21.8	22.0	17.8	14.1
	(422.6)	(9.8)	(517.9)	(695.0)
	+0.54	−0.13	+0.27	+0.33
Cyclops vernalis	65.1	49.6	24.2	13.4
	(149.0)	(7.1)	(203.3)	(161.1)
	−0.43	−0.60	−0.33	−0.35
Diaptomus siciloides	22.7	16.4	6.7	8.4
	(33.4)	(0.9)	(25.2)	(39.8)
	−0.59	−0.83	−0.64	−0.68
D. clavipes	0.7	0.5	0.1	0.1
	(2.3)	(0.2)	(0.4)	(2.8)
	−0.20	−0.20	−0.33	0.00
Length range of bullheads (mm)	37–58	50–66	61–87	62–87
Number of bullheads examined	50	50	50	50

most abundant (14–22/liter) and smallest cladoceran (<1.2 mm) available to young-of-the-year black bullheads. Although this species comprised a major portion of the diet of the young-of-the-year, selectivity indices indicated that it was not highly selected by bullheads ranging from 50 to 87 mm total length, and that it would probably have been avoided in the presence of a dense *Daphnia* population.

The negative selectivity indices for Copepoda indicate why copepods and small cladocerans may predominate in lake plankton populations in the late summer and fall. *Cyclops vernalis* represented a small crustacean (<1.8 mm) that was consistently negatively selected, and *Diaptomus siciloides* a small crustacean (<1.3 mm) that was capable of avoiding fish predation. *Diaptomus clavipes* represented a large crustacean (<2.5 mm) capable of avoiding fish predation. Calanoid copepods are capable of rapid movement and diversionary tactics. This may be observed when one attempts to remove *Diaptomus* spp. from an aquarium with a pipette. Langford (1953) reported that *Diaptomus* avoids capture by plankton pumps whereas *Cyclops* is positively selected. The bigmouth buffalo is an indiscriminate planktivore in Lake Poinsett that filters planktonic organisms by the use of gill rakers and accessory teeth. Calanoid copepods consistently avoid it, and selectivity indices for *Diaptomus* are at the extreme negative end of the scale (Starostka and Applegate 1970).

The black bullhead preyed upon a wide range of food sources in the limnetic, littoral, and benthic zones of Lake Poinsett and seasonal differences in diet occurred in response to availability of prey species. All length-groups of black bullheads were selective for the largest cladocerans available, *L. kindtii*, *D. pulex*, and *D. g. mendotae*. The black bullhead in Lake Poinsett was primarily a selective planktivore throughout its life history.

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Changes in Blood Chemistry and Blood Cell Morphology in Coho Salmon (*Oncorhynchus kisutch*) Following Exposure to Sublethal Levels of Total Residual Chlorine in Municipal Wastewater

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Yearling coho salmon (*Oncorhynchus kisutch*) were exposed for 12 wk to either 0.3, 1.1, or 3.6% chlorinated municipal sewage treatment plant effluent with seawater diluent (average salinity 28‰) under continuous flow conditions. The maximum safe concentration of effluent lies between 0.3 and 1.1% (average total residual chlorine ($TRCl_2$) content 0.003 and 0.009 mg/liter, respectively). Effluent concentrations of 0.3% produced no discernible effects on the fish. Concentrations of 1.1 and 3.6% (average content of $TRCl_2$ 0.030 mg/liter) resulted in reductions of hemoglobin and hematocrit to levels indicative of anemia. Observations of the erythrocytes revealed lysed and degenerating cells, increased numbers of circulating immature cells, and abnormal cells. These hematological effects are attributed to the oxidative nature of $TRCl_2$.

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Nous avons exposé durant 12 semaines des saumons coho (*Oncorhynchus kisutch*) de l'année à des concentrations de 0.3, 1.1 ou 3.6% d'effluent chloré d'usine de traitement d'égoûts municipaux dilué avec de l'eau de mer (salinité moyenne de 28‰) et à écoulement continu. La concentration sûre maximale de l'effluent se situe entre 0.3 et 1.1% (contenu en chlore résiduaire total moyen (Cl_2RT) de 0.003 et 0.009 mg/litre respectivement). Les teneurs en effluent de 0.3% ne produisent aucun effet discernable chez les poissons. Des teneurs de 1.1 et 3.6% (contenu moyen en Cl_2RT de 0.030 mg/litre) abaissent l'hémoglobine et l'hématocrite à des niveaux indiquant l'anémie. L'examen des érythrocytes révèle des cellules lysées et en voie de dégénération, un plus grand nombre de cellules immatures en circulation et des cellules anormales. Nous attribuons ces effets hématologiques à la nature oxydante du Cl_2RT.

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THE chlorination of sewage treatment plant effluent is a measure widely practiced to prevent contamination of receiving waters by pathogenic bacteria and viruses. Chloramines (combined residual chlorine) are formed upon combination of the chlorine with the ammonia in the wastewater. Compared with free residual chlorine, combined residual chlorine is reported to be as toxic, or slightly less toxic, to fish (Doudoroff and Katz 1950; Merkens 1958; Rosenberger 1971) but to be more persistent in the receiving waters. The effects of residual chlorine on freshwater biota have been studied for some time (Brungs 1973), but relatively little research has been done to determine its effects on marine organisms. The sublethal toxicity of chlorinated effluent in marine waters to coho salmon (*Oncorhynchus kisutch*) was determined and safe effluent concentrations were established under conditions used in this investigation. Additionally, the hematological methods by which effluent toxicity is measured were evaluated.

The work was conducted at the Municipality of Metropolitan Seattle's West Point Treatment Plant, located north of Elliott Bay on Puget Sound. An average of 380,000 m^3/day of wastewater from domestic and industrial sources is

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given primary treatment, chlorinated, and discharged via a diffuser outfall 1110 m offshore at a depth of 70 m.

Materials and Methods

Yearling coho salmon (average wet weight, 29 g) were obtained from the Washington State Department of Fisheries hatchery at Puyallup and divided into eight groups of 180 fish each. Oregon Moist Pellets were fed twice daily on weekdays (2% of body weight/day) and once on Saturdays (1% of body weight/day). Feed was withheld 48 h prior to either blood or weight sampling.

Acclimation and testing were conducted in 400-liter circular fiberglass tanks provided with a continuous flow of effluent and sea water by PVC pipe. Effluent was taken near the entrance to the plant outfall line by a metal-free pump and diluted in a closed dilution system with sea water (average salinity 28‰) obtained from Puget Sound by a submersible stainless-steel pump. Flow rate to each tank was 4 liters/min (95% replacement of tank content in 5 h), with a maximum measured error of less than 2% of the nominal tank effluent concentration. Feces and excess feed were flushed and siphoned daily from the tanks and periphyton was brushed and siphoned weekly from both the tanks and diluter. All fish-handling equipment was routinely disinfected in a 500 mg/liter solution of Roccal.

The experiment consisted of three parts, during which duplicate groups of fish underwent: 1) a 2-wk acclimation to sea water (salinity increased 7‰ daily over a 4-day period) and the test environment, 2) a 12-wk exposure to either the control (0%) or one of three test solutions (either 0.3, 1.1, or 3.6% effluent), and 3) a 4-wk recovery period in 100% sea water.

Blood was sampled from 10 fish randomly taken weekly or biweekly between 0–12 wk and at 16 wk from each group except the one in 1.1% effluent concentration. In this group blood was sampled from five fish during week 7, 9, 11, 12, and 16. The fish were anesthetized in a continuously aerated solution of M.S. 222 (80 mg/liter of sea water), and blood was taken from the severed caudal peduncle. Smears were prepared immediately from the unheparinized blood, air dried, fixed in absolute methanol, and stained with Giemsa. Five hundred erythrocytes from each smear were examined at random under an oil-immersion microscope and classified as either mature or immature, according to morphology and staining propetries. Concurrently, the percentages of various types of leukocytes were estimated from preliminary screening counts of 25, followed by counts of 100 if necessary. Blood was collected in a volumetric capillary tube rinsed with 10 mg/ml sodium heparin solution, and the hemoglobin (Hb) and methemoglobin (MHb) were measured according to the Cyanmethemoglobin method and to Lukeman (1959), respectively. Blood was also collected in heparinized microhematocrit tubes and centrifuged at 11,500 rpm for 5 min, and the hematocrit (Ht) was measured with a capillary tube reader. Significant differences in blood measurements were determined by the nonparametric test of Lord (1947). Fish weights (measured week 2, 4, . . . 12, 16) were expressed as the average wet weights of random samples of about 25 fish from the test and control groups.

External and internal examinations of the 391 sample fish revealed signs of abnormalities and/or disease in 14 fish. Data from these 14 fish were used unless the values fell outside the range established by fish sampled from a duplicate tank. Mortality during the 16 wk was 4%, not including that ascribed to effluent toxicity.

Water quality factors in midtank grab samples were analyzed according to *Standard Methods* (American Public Health Association 1971) except ammonia, which was measured by the method of Saxton and Zadorojny (1973) (Table 1). The total residual chlorine ($TRCl_2$) concentrations in the tanks were estimated from calculated values, which agreed well with the data from an analysis of grab samples by a modified amperometric titration procedure with a detection limit of 0.01 mg/liter. Because of high ammonia content, the amount of free residual chlorine in the effluent was negligible and the measured $TRCl_2$ consisted of combined residuals, probably in the monochloramine form. Differences in salinity between tanks averaged 1%, whereas differences in pH were very slight at any one time. The average tank surface water temperature was 13.7 C and the maximum and minimum temperatures were 18.1 and 11.8, respectively, which approximated the temperature regime of ambient sea water. The effluent content of $TRCl_2$ was monitored 24 h/day by a strip-chart recorder calibrated by the amperometric titration method (Table 2). The effluent content of heavy metals was analyzed daily on acidified 24-h composite samples with the aid of an IL453 atomic absorption spectrophotometer.

Results

The fish exposed to 0.3% effluent for 12 wk were not significantly ($P = 0.05$) affected, but those fish that were exposed to 1.1% effluent and the survivors of exposure to 3.6% effluent showed signs of sublethal stress. Blood Hb and Ht declined, the number of circulating immature erythrocytes increased, and the condition of all erythrocytes generally deteriorated. These fish generally showed symptoms of hemolytic anemia, and those exposed to 3.6% additionally showed evidence of microcytosis. Mortality was 40% at this test concentration.

Tests (Buckley and Matsuda 1972) at this laboratory have established that the toxicity of the effluent is principally determined by $TRCl_2$ content. Time plots of Hb concentration and percent immature erythrocytes in the present study showed that the magnitude of stress to the

TABLE 1. Water quality of various concentrations of a chlorinated municipal sewage treatment plant effluent (seawater diluent).

% Effluent	Avg[a]	SD	Maximum	Minimum
	Total residual chlorine,[c] mg/liter			
0[b]	0.000 (3)			
0.3	0.003 (78)	0.002	0.008	0.000
1.1	0.009 (78)	0.008	0.029	0.000
3.6	0.030 (78)	0.025	0.094	0.000
	$NH_4 - N$[d] $+ NH_3 - N$, *mg/liter*			
0[b]	0.059 (76)	0.020	0.114	0.027
0.3	0.069 (76)	0.027	0.194	0.027
1.1	0.126 (61)	0.054	0.295	0.027
3.6	0.376 (74)	0.158	0.598	0.027
	$NH_3 - N$[d] *(un-ionized), mg/liter*			
0[b]	0.002 (66)	0.001	0.005	0.001
0.3	0.002 (65)	0.001	0.007	0.001
1.1	0.004 (55)	0.003	0.015	0.001
3.6	0.010 (65)	0.007	0.038	0.001
	Dissolved oxygen, mg/liter			
0[b]	6.7 (84)	1.4	10.5	4.3
0.3	6.7 (84)	1.5	10.2	4.5
1.1	7.0 (67)	1.6	10.9	4.7
3.6	7.1 (82)	1.5	11.3	5.5
	Total alkalinity, mg/liter as $CaCO_3$			
0[b]	97.8 (22)	3.7	103.2	92.0
0.3	97.8 (22)	3.6	104.0	92.5
1.1	96.4 (17)	3.7	104.0	91.1
3.6	97.2 (21)	3.9	103.0	91.1
	Turbidity, JTU			
0[b]	1.0 (20)	0.7	3.8	0.5
0.3	0.9 (20)	0.4	2.3	0.5
1.1	1.4 (15)	0.6	2.4	0.2
3.6	2.3 (19)	0.7	3.6	0.8

[a]Average of (N) individual tests.
[b]Control.
[c]Daily average.
[d]Corrected to activity.

fish was proportional to the effluent concentration to which they were exposed (Fig. 1a, b). Furthermore, physiological changes in the fish, as measured by these parameters, correlated with fluctuations in effluent $TRCl_2$ (Fig. 1c).

The percentage of immature erythrocytes appears to be more sensitive to those fluctuations than the Hb fraction, especially at the 3.6% effluent level of exposure. The numerical increase in circulating immature erythrocytes after 1 wk of exposure is indicative of compensatory erythropoiesis, probably resulting from oxidant damage to circulating erythrocytes (Eaton et al. 1973) by $TRCl_2$. The immature erythrocytes consisted of some or all of the following developmental stages: pronormoblast, basophilic, polychromatophil, and orthochromatophil normoblasts (nomenclature from Wintrobe 1946). With increasing stress, more primitive forms appeared. Hb levels in the fish exposed to 1.1 and 3.6% effluent were significantly reduced ($P = 0.05$) from the controls by the end of the 2nd wk and averaged about 75 and 45% of control levels, respectively, during the remainder of the test.

In addition to normal and abnormal erythrocytes, lymphocytes, segmented neutrophils, thrombocytes, and nuclear smudges, or "basket cells," were seen in the smears. Differential counts of leukocytes did not show a definite trend in relation to effluent concentration. In the fish exposed to 1.1% effluent immature cells were generally orthochromatophils. Erythrocytes showed clump-

TABLE 2. Water quality of West Point Treatment Plant effluent during the 12-wk toxicity test.

Parameter	Avg[a]	SD	Maximum	Minimum
Total residual Cl_2,[b] mg/liter	0.840 (78)	0.475	2.61	0
$NH_4 - N + NH_3 - N$, mg/liter	11.48 (35)	4.77	19.75	0.05
$NH_3 - N$ (un-ionized), mg/liter	0.173 (32)	0.438	1.96	0.001
Total alkalinity,[c] mg/liter	106.7 (11)	15.3	134.1	90.7
pH			9.02	6.45
Dissolved oxygen, mg/liter	0.7 (39)	0.8	2.8	0
$NO_2 - N$, mg/liter	0.05 (35)	0.05	0.17	0.01
Turbidity, JTU	41 (10)	16	83	25
Temperature, C	20.1 (108)		22.4	19.3
Metals, mg/liter				
Cd	0.008 (80)	0.005	0.019	0.004
Cr	0.07 (80)	0.03	0.13	0.01
Cu	0.20 (80)	0.04	0.36	0.11
Ni	0.07 (80)	0.02	0.16	0.05
Pb	0.1 (80)	0.1	0.2	0.1
Zn	0.370 (80)	0.009	0.720	0.190

[a]Average of (N) individual tests.
[b]Daily average.
[c]As $CaCO_3$.

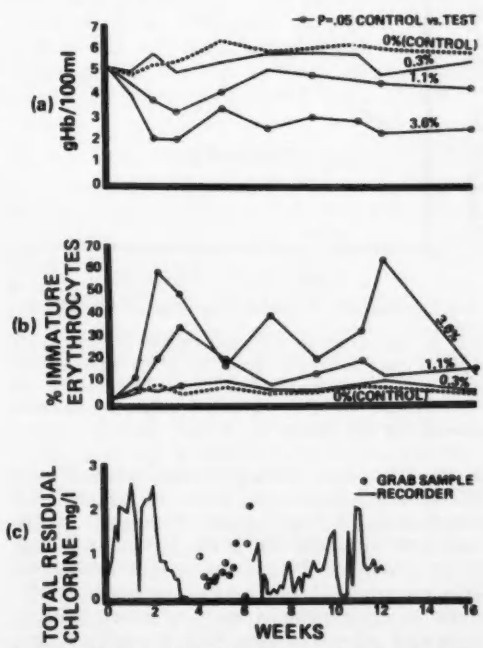

FIG. 1. Relationship between a) average total hemoglobin, b) average percent immature erythrocytes in yearling coho salmon (*Oncorhynchus kisutch*), and c) total residual chlorine in 100% effluent during period of exposure to various percentages of chlorinated effluent in seawater diluent (week 0–12) and during period of recovery in 100% sea water (week 12–16).

ing of chromatin and occasional lysis of the nuclear membrane. In the blood of fish exposed to 3.6% effluent orthochromatophils were common, polychromatophils less common, and basophilic normoblasts and pronormoblasts infrequent. Both mature and immature erythrocytes had a "ragged," vacuolated appearance and also showed clumping of chromatin. Furthermore, abnormal sizes and shapes of erythrocytes including microcytes, tear drops, and dividing cells were common (Fig. 2). In contrast, the blood of the control fish contained few orthochromatophils and nuclei of mature erythrocytes had uniform fine-grained chromatin (Fig. 3). "Basket cells" occurred in the smears in proportion to the level of effluent

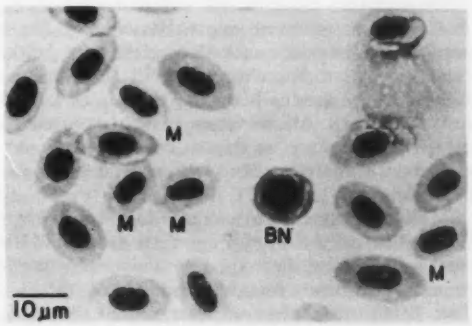

FIG. 2. Erythrocytes with "ragged," vacuolated cytoplasm in fish exposed to 3.6% effluent (seawater diluent) for 12 wk. Basophilic normoblast (BN) and microcytes (M) exemplify the blood dyscrasia.

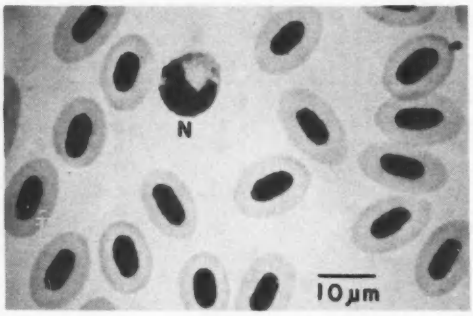

FIG. 3. Erythrocytes from control fish after 12 wk in 100% sea water. A neutrophil (N) is also shown.

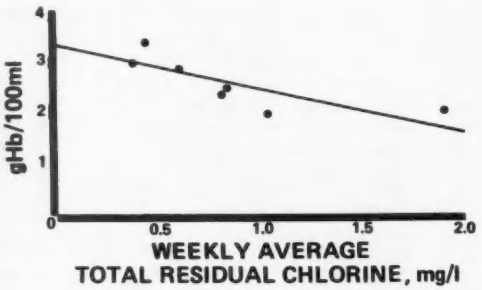

FIG. 4. Relationship between weekly average total residual chlorine concentration in 100% effluent and total hemoglobin in coho salmon exposed to 3.6% effluent (seawater diluent) for 12 wk.

concentration; the trend appeared to be related to increased cell fragility or number of senile cells.

Four weeks after termination of exposure, Hb values of fish previously exposed to 3.6% effluent had improved slightly and immature erythrocyte counts were reduced. Morphologically, erythrocytes also reflected a recovery. The mature erythrocytes appeared more uniform and compared favorably with those of control fish, except for a patchy lightness in the cytoplasm, which indicated reduced Hb content.

The weekly average $TRCl_2$ measurements in the 3.6% effluent vs. the average Hb values and the average immature erythrocyte counts for the test fish were fitted by the least squares method and showed correlation coefficients of -0.80 and 0.74, respectively (Fig. 4, 5). Data from the first sampling period (week 1) were not included because of the initial lag in response to the $TRCl_2$ and possible hourly fluctuation in effluent content of $TRCl_2$.

Hematocrit values of fish exposed to 1.1 and 3.6% effluent showed approximately the same pattern as Hb: a decrease during the first 3 wk, followed by an improvement until the 5th wk, followed by a gradual decrease to the end of the test (Table 3). Microcytosis in fish exposed to 3.6% effluent may in part account for the decrease in hematocrit both during and after exposure.

MHb was expected to increase in test fish when the ability of the erythrocytes to reduce MHb was exceeded by the oxidative action of chloramines as previously found by Eaton et al. (1973). The MHb concentration averaged 0.18, 0.24, 0.25, and 0.22 g/100 ml in fish exposed to 0% (control), 0.3, 1.1, and 3.6% effluent, respectively. Although test fish had significantly ($P = 0.05$) higher MHb levels than control fish 33%

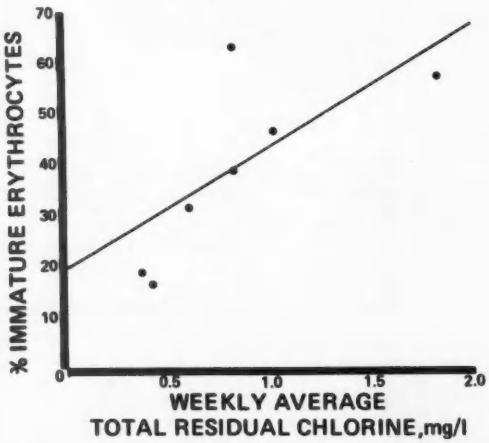

FIG. 5. Relationship between weekly average total residual chlorine concentration in 100% effluent and percent of immature erythrocytes in circulation in coho salmon exposed to 3.6% effluent (seawater diluent) for 12 wk.

of the time, the biological significance of the difference is questionable when the proportional reduction in the oxygen-carrying capacity of the blood, as measured by MHb percent of total Hb, is considered. Fish in the group experiencing 40% mortality (3.6% effluent) averaged 8.2% MHb. In comparison, a study of nitrite toxicity to chinook salmon showed 40% mortality of fish in which MHb averaged 6.7 g/100 ml, or an average of 80% MHb of total Hb (Smith and Williams 1974). The toxic action of $TRCl_2$ is not due to methemoglobinemia since toxicity was manifest at a concentration of $TRCl_2$ below which there was biologically significant MHb formation.

Weight and response to feeding were also

TABLE 3. Average hematocrit percentage in coho salmon (*Oncorhynchus kisutch*) exposed to various concentrations of a chlorinated municipal sewage treatment plant effluent.

Time (*wk*)	1	2	3	5	7	9	11	12	16[a]
0%[b]	29	32	32	45	33	34	33	34	33
0.3%	35[c]	37	33	40	37	37	32	32	35
1.1%	33	28	27[c]	34[c]	33	34	29	34	29
3.6%	27	21[c]	23[c]	33[c]	25[c]	25[c]	26[c]	23[c]	21[c]

[a]Four weeks after termination of exposure to effluent.
[b]Control.
[c]$P = 0.05$ control vs. % effluent.

affected by effluent concentration. The fish exposed to 3.6% effluent lost an average of 4 g by the end of the 16th wk, whereas all the other fish had gained in excess of 10 g. Throughout the study, fish in 0 and 0.3% effluent generally responded vigorously when food was presented. Fish in 1.1% effluent responded slightly less vigorously, and those in 3.6% effluent responded poorly to food. The same differential feeding response was observed throughout the 4-wk recovery period; the fish in 3.6% effluent never recovered their interest in food.

Discussion

The anemia in fish exposed to 1.1 and 3.6% effluent concentrations was identified as hemolytic on the basis of observed lysis of the nuclear and cytoplasmic membranes of erythrocytes. Attempts to trace the products of the breakdown of hemoglobin by measurements of the plasma hemoglobin and bilirubin were inconclusive. Anemia can be caused by a number of pathological conditions. In this instance it appears to be a characteristic response to either oxidants (Whitmore 1965) or agents affecting osmotic stability (Smith et al. 1971), and not a specific response to the sublethal toxicity of $TRCl_2$. The stages of anemia were similar to those noted in juvenile spring chinook salmon (*Oncorhynchus tshawytscha*) fed rancid diets deficient in vitamin E (Whitmore 1965).

A question that arises when one tests for sublethal toxicity is whether the changes measured in a test animal are deleterious or are an indication of successful acclimation to environmental change. Physiological changes indicating impaired respiration are not generally considered signs of successful acclimation (Sprague 1969). A prolonged reduction in Hb is deleterious to oxygen transport. In addition, dyscrasia and degeneration of the erythrocytes are pathological conditions. None of these changes can be considered indications of successful acclimation.

In this work $TRCl_2$ averaged 0.003, 0.009, and 0.030 mg/liter in 0.3, 1.1, and 3.6% effluent, respectively. In a 23-day study of the effect of residual chlorine on coho salmon in sea water, Holland et al. (1960) found a critical limit of about 0.05 mg/liter. In our study 40% mortality occurred when fluctuating concentrations averaging 0.030 mg/liter reached values up to 0.094 mg/liter during the early part of the test.

There are no comparable studies of the sublethal toxicity of residual chlorine in sea water; therefore, studies in freshwater are cited. Dandy (1972) observed depressed activity in brook trout (*Salvelinus fontinalis*) exposed to residual chlorine concentrations as low as 0.005 mg/liter for 7 days. He considered this a sublethal level, below which the threshold for the depressant response must occur. In a 21-wk test with fathead minnows (*Pimephales promelas*), the highest $TRCl_2$ concentration with no significant effect on egg production was 0.0165 mg/liter (Arthur and Eaton 1971). Sprague and Drury (1969) reported significant avoidance reactions in rainbow trout (*Salmo gairdneri*) to concentrations of 0.01 and 1.0 mg/liter and a preference for an intermediate concentration of 0.1 mg/liter, lethal in about 4 days. The authors surmised that deadened sense organs may have kept the trout in this "trap."

Static- and continuous-flow toxicity tests (Buckley and Matsuda 1972; Buckley 1974) at this laboratory defined 96-h TL50's of 0.1 and 0.07 mg/liter, respectively, for chlorinated effluent in sea water. Use of an application factor of 0.1 (Environmental Protection Agency 1973) for free residual chlorine in sea water results in maximum acceptable concentrations (MAC) of 0.01 and 0.007 mg/liter for static- and continuous-flow tests, respectively. The results of the continuous-flow test agree with the values obtained in the present work: the inaffective level, or MAC, lies

between 0.003 and 0.009 mg/liter $TRCl_2$, based on averages of fluctuating concentrations. In comparison, a recent literature review (Brungs 1973) recommended that the residual chlorine content should not exceed 0.002 mg/liter for the protection of sensitive freshwater species in areas continuously receiving chlorinated wastes. An Environmental Protection Agency publication (1973) proposed a maximum standard of 0.01 mg/liter for free residual chlorine in marine waters, a standard that is confirmed by results from this work.

Tests by Abram (1967) and Brown et al. (1969) indicate that when fish are exposed to fluctuating concentrations of toxicants, mortality may be predicted from the effect of the average concentration. However, the effects of fluctuating sublethal concentrations may not be predicted from the effects of the average concentration (Sprague 1970). In this work fluctuating concentrations of residual chlorine elicited levels of response from the fish which confirm the validity of the standards set for this toxicant in sea water. However, these responses were not compared with responses elicited by uniform concentrations. Because fluctuations are expected in field situations, the relationship between responses to uniform concentrations (upon which standards are often based) and those to fluctuating concentrations merits further investigation.

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Year-Class Formation in the Walleye (*Stizostedion vitreum vitreum*) Population of Oneida Lake, New York, 1966–73[1]

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FORNEY, J. L. 1976. Year-class formation in the walleye (*Stizostedion vitreum vitreum*) population of Oneida Lake, New York, 1966–73. J. Fish. Res. Board Can. 33: 783–792.

Abundance of eight successive year-classes of walleyes (*Stizostedion vitreum vitreum*) was measured at intervals from hatching into the second year. Each year-class arose from an initial stock of 12–18 billion eggs. Population of pelagic larvae was augmented in some years by the release of hatchery-reared larvae but the effect on year-class size was obscured by mortality after young became demersal. Decrease in number of fingerlings was attributed to predation by older walleyes. Intensity of predation was influenced by abundance of alternate prey and duration of cannibalism by growth of young walleyes. Five year-classes that were monitored through age 4 contributed 12,000–478,000 walleyes to the adult stock.

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Nous avons mesuré l'abondance de huit classes d'âge successives de dorés (*Stizostedion vitreum vitreum*) à divers intervalles entre l'éclosion et la deuxième année. Chaque classe d'âge était issue d'un stock initial de 12–18 milliards d'œufs. Certaines années, la mise en liberté de larves élevées en pisciculture a grossi la population de larves pélagiques, mais l'effet sur l'abondance des classes d'âge a été masqué par les mortalités survenues après que les jeunes fussent devenus démersaux. On attribue à la prédation par les autres dorés la diminution des alevins de la grosseur du doigt. L'intensité de la prédation dépend de l'abondance de proies alternatives et de la durée du cannibalisme, elle-même fonction de la croissance des jeunes dorés. Cinq classes d'âge suivies jusqu'à l'âge 4 inclusivement contribuèrent de 12,000–478,000 dorés au stock d'adultes.

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WIDE fluctuations in size of walleye (*Stizostedion vitreum vitreum*) year-classes are common (Smith and Krefting 1954; Pycha 1961) but the causes are usually obscure. Since each cohort arises from a different parent stock and is subject to a unique set of mortality coefficients during its formation, the difficulty in identifying factors controlling recruitment is not surprising. The problem becomes more tractable if abundance of successive cohorts is measured at sequential life stages from hatching to recruitment. This approach is especially applicable to Pacific salmon (Paulik and Greenough 1966) which exhibit well-defined life stages but the technique has also been used to examine recruitment of fish in inland waters (Miura 1965).

In Oneida Lake, year-classes of walleyes produced in 1966–73 were followed through the egg, larval, fingerling, and yearling stages. Abundance was estimated at each stage and relative survival of cohorts compared. Apparent intensity of mortality experienced by different cohorts during comparable periods of development were related to variables that were expected to influence mortality. The objective was to delineate the period when year-class size was established and identify factors that contributed to variation in year-class abundance.

Oneida is a shallow eutrophic lake in central New York with a surface area of 207 km^2 and a mean depth of 6.8 m. The lake is usually homothermal, but brief periods of temperature and oxygen stratification occasionally develop in summer (Greeson 1971). The walleye and yellow perch (*Perca flavescens*) are the predominant species constituting about 70% of the biomass of fish captured in experimental gillnets, trapnets, and trawls. These two species constitute a simple predator–prey association with walleye feeding primarily on young and yearling perch.

Walleyes spawn in April and the eggs are scattered over gravel and rubble in the lake and tributary streams. Eggs begin hatching in late April

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or early May and hatching is complete within 10 days. Larvae, which average 7.5 mm at hatching, are pelagic until late June (Houde and Forney 1970). Transition to the demersal mode begins in June when young attain a mean length of 35 mm and is usually complete by mid-July when fingerlings average 60 mm. Both fingerlings and adults are widely distributed, occupying both shoal and deeper mud-bottom areas during late summer and fall. Female walleyes mature at age 4 or 5 at a length of 360–400 mm and males at a somewhat younger age and smaller size (Forney 1965a).

Materials and Methods

Description of Gear

Walleye larvae were collected in fiberglass samplers (Miller 1961) with a 14-cm diameter body and a 10-cm orifice. Opaque dark green samplers were used in 1966–68 and translucent, unpigmented samplers in subsequent years. Samplers were equipped with nets of monofilament nylon with a .55-mm aperture. Two samplers were attached to each of two cables with 7-kg depressors and towed from a boat propelled with twin 40-hp motors. A Clarke–Bumpus flowmeter was mounted on a cable between two samplers to determine the volume filtered. The flowmeter was calibrated by towing over a known distance and it was assumed all water encountered was filtered (Miller 1961). After walleyes became demersal in mid-July abundance of fingerlings was estimated from the catch in an otter trawl with a 5-m headrope and a 5.5-m footrope. Wings and body were constructed of 38-mm stretch nylon with 13-mm mesh nylon cod-end. The net was fished with double 1-m bridles attached to flat boards with an area of .32 m^2.

Sampling Design

Larval abundance was estimated from the catch at randomly selected locations within three strata. The strata encompassed the volume of water between the 2–4-m contours, between the 4–6-m contours, and the area within the 6-m contour to a depth of 6 m. Although the sampling space excluded the zone below 6 m and between shore and the 2-m contour, it bounded most of the population. A single walleye was captured in 80 samples taken at depths of 7–12 m in 1968–71. Risk of damage prevented use of Miller samplers in water less than 2 m deep but walleye larvae were seldom captured in meter nets towed between shore and the 2-m contour. In most years samples were collected at 20 randomly selected stations in each stratum. At each station four Miller samplers were towed simultaneously at a speed of 3.6 m/s (8 mph) parallel to a depth contour and the catch pooled. Depth of samplers was selected at random with two above and two below the median depth to a maximum depth of 6 m. Duration of the tow was generally 5 min at stations located in the two inshore strata and 7–½ min in the offshore stratum. Abundance was estimated when walleyes averaged 9–10 mm and were about 10 days old. At this size walleye larvae are unlikely to avoid high-speed samplers (Noble 1970) and the length distribution of the catch was usually normal which indicated most eggs had hatched and recruitment to the pelagic stage was complete. Estimates of the larval population followed the method of complete enumeration of selected subsamples outlined by Regier and Robson (1967). Population of larvae in each stratum was the product of the mean catch and number of sampling spaces. Number of sampling spaces was determined by dividing the volume of a stratum by the mean volume strained for four samplers. Stratum estimates were summed to determine the total population. Variance was calculated for the population in each stratum and stratum variances summed to estimate variance for the total population (Cochran 1963). Independent estimates of the population and other internal evidence confirmed that the procedure for estimating larval abundance was reliable (Forney 1975a).

Abundance of fingerlings was estimated from the catch in trawls fished at weekly intervals beginning when walleyes averaged 60 mm in late July and terminating in late October. Hauls were made during the day at 10 sites located in water 5–12 m deep. Trawling speed was 3.4 km/h and a standard 5-min haul swept about 1000 m^2 based on the width of the mouth opening while the net was under tow. Natural logarithm of the mean weekly catch of fingerlings was regressed on date of capture and the relative abundance of cohorts on August 1 and October 15 read from the fitted regression. It was assumed that unweighted mean catches taken during a short interval of time were proportional to the total population and the decline in catch was in index of mortality occurring between July and October. Mortality was taken as occurring in a manner that could be modeled by a continuous Poisson process with parameters directly proportional to elapsed time. The expected outcome of such a process is a continuous exponential function as is commonly assumed to hold for fish populations. The logarithmic transformation of means catches, then, is expected to yield a straight line with deviations from regression showing no trend with time.

Yearling and Adult Stage

Yearling walleyes were captured in trawls fished for fingerlings. Abundance of yearlings was estimated from the mean catch per effort (c/f) during the period from late July through October about 16 mo after hatching.

Population of adult walleyes was determined in 1966–74 by mark and recapture following procedures developed in earlier studies (Forney 1967). Population estimates were made by age-groups that provided quantitative estimates of size of individual year-classes recruited to the adult stock at age 4.

Data on year-class size together with information on fecundity and maturity were used to estimate egg

production. Annual egg production was the sum of estimates for age-group 4 and older. Mean weight ($\bar{w}$) of females of each age was monitored and

$$\text{Fecundity} = 70{,}600\ \bar{w}\ (\text{kg}) - 7{,}900$$

The weight–fecundity regression was based on volumetric measurement and counts of aliquots of eggs from ovaries of 36 walleyes that ranged between 0.4 and 1.8 kg. Egg production was the product of fecundity and numerical size of a year-class as determined by mark and recapture. All age 5 and older females and about 30% of the age 4's were mature and a sex ratio of 1:1 was assumed. Egg production may be slightly overestimated since 3–15% of the adult females usually fail to form eggs (Forney 1965a) and an additional 2–3% of the females were netted and eggs taken for hatchery culture.

Formation of Year-Classes

Walleyes produced 12–18 billion eggs (580,000–870,000/ha) in 1966–73 (Table 1). Small differences in egg production had little discernible effect on number of larvae. However, there was a dramatic decrease in numerical size of cohorts between the egg and larval stage. Highest density of larvae was 2,340/ha in 1970 and the lowest 319/ha in 1971. Comparison of egg production and larval abundance suggests mortality consistently exceeded .995 between spawning and the time cohorts attain a mean length of 9–10 mm.

A high proportion of the larvae in 1966–68, 1970, and 1972 probably originated from the release of 1–3-day-old hatchery-reared larvae. Eggs from walleyes netted in Oneida Lake were incubated in water from Scriba Creek, a tributary, and stocked at about the time eggs spawned in the lake begin to hatch. Populations of larvae approximately 10 days after larvae were released ranged from 22 to 48 million (Table 1). In 1969, 1971, and 1973 when all larvae originated from natural spawning the number of larvae was 6–10 million at a comparable time and stage of development.

TABLE 1. Annual egg production, number of larvae stocked, and estimated number of larvae 8–12 days after eggs hatched.

Year	Egg production ($\times 10^9$)	No. larvae ($\times 10^6$)		
		Stocked	Population	
			Estimated no.	SE
1966	18	194	32.8	4.9
1967	14	157	21.9	3.8
1968	12	180	40.5	7.6
1969	15	0	10.2	2.6
1970	14	310	48.4	5.4
1971	12	0	6.6	1.6
1972	13	214	29.2	8.5
1973	12	0	8.2	1.6

Relative abundance of year-classes was substantially modified by mortality between May when young were pelagic and August when cohorts were demersal (Table 2). Mortality increased variation in year-class size and obscured initial differences in larval abundance. Although stocking was successful in augmenting larval populations, effect of stocking on year-class size would be difficult to detect if estimates of larval abundance were not available. A low correlation of 0.10 between number of larvae stocked and fingerling catch on August 1 would lead to the conclusion that stocking was ineffective. However, in years when cohorts were augmented most of the fingerlings in August were of hatchery origin unless natural and stocked larvae experience differential mortality during the post-larval or later stages of development.

Ranking of year-classes continued to change during the demersal stage. Catch of fingerlings in trawls declined rapidly during summer and fall of 1966, 1969, 1970, and 1972 (Fig. 1). In contrast, catches in 1967, 1968, and 1971 showed only a modest seasonal decrease. These trends in mortality which developed during late summer caused further divergence in year-class size. By October trawl catches indicated a 185-fold range in size of cohorts compared to a 7-fold range at the larval stage.

By age 1 the 1967, 1968, and 1971 cohorts were clearly the dominant year-classes among the eight studied. Relative size of year-classes was fixed by midsummer of the second year as shown by close agreement between the catch of yearlings in trawls and population estimates at age 4. The strong 1968 year-class contributed about 478,000 walleyes to the fishery and the 1969 year-class only 12,000.

Factors Regulating Year-Class Formation

Numerical size of walleye year-classes recruited to the fishery was determined by initial abundance of larvae and different rates of mortality acting on successive stages of development. Changes in relative abundance of year-classes between successive stages of development were examined to identify causes of mortality and their relative importance in determining year-class strength.

Egg and Larval Stage

Over 99% of the mortality experienced by walleye year-classes occurred before larvae were 9–10 mm long and most of the loss probably

TABLE 2. Abundance of the 1966–72 year-classes of walleyes (*Stizostedion vitreum vitreum*) at intervals following hatching.

Year-class	Fry population ($\times 10^6$)	Mean c/f in trawls: 0 Aug. 1[a]	0 Oct. 15[a]	1 July–Oct.	Estimated no. at age 4 ($\times 10^3$)
1966	32.8	3.65	0.42	0.08 (140)[b]	24
1967	21.9	9.61	7.91	0.72 (140)	198
1968	40.5	22.41	20.38	3.89 (130)	478
1969	10.2	10.82	4.63	0.03 (140)	12
1970	48.4	6.53	1.72	0.16 (160)	55
1971	6.6	5.70	4.07	3.43 (110)	—
1972	29.2	5.79	0.40	0.04 (140)	—
1973	8.2	0.26	0.11	0.09 (140)	—
Coefficient of variation	0.22	0.29	0.48	0.54	0.57

[a]Predicted from regressions in Fig. 1.
[b]Number of hauls.

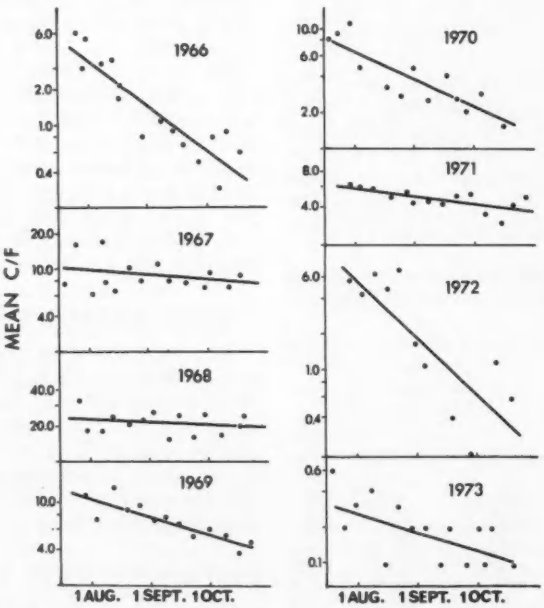

FIG. 1. Mean catch of young walleyes (*Stizostedion vitreum vitreum*) at 10 sites sampled by trawling at approximately weekly intervals in 1966–73.

TABLE 3. Population of larval walleyes in Oneida Lake estimated from successive surveys in 1968 and in 1970–73.

Year	Date	Estimated population		Mean length (*mm*)
		No. ($\times 10^6$)	SE ($\times 10^6$)	
1968	May 8–10	40.5	7.6	9.0
	May 13–14	32.7	5.6	9.4
1970	May 11–13	48.4	5.4	9.0
	May 23–24	47.0	7.9	11.0
1971	May 25–27	6.6	1.6	9.6
	June 1–3	6.4	3.6	11.0
1972	May 23–24	29.2	8.5	10.0
	May 29–31	33.1	5.5	13.8
1973	May 11–12	8.2	1.6	9.0
	May 23–25	4.6	1.1	10.1

occurred during the egg stage. Number of eggs on shoals in the lake decreased rapidly following spawning and few eyed eggs could be found. Onshore winds and accompanying waves scoured the shoals and destroyed or dislodged most eggs (Forney 1965b). Eyed eggs were common in tributaries but density of larvae near the mouths of tributaries was often lower than in the open lake (Houde and Forney 1970). Yearling perch frequently invaded the lower reaches of tributaries in the spring and larval walleyes were found in stomachs of perch captured in Scriba and Fish creeks. Bean (1912) also reported perch predation on walleye fry in Scriba Creek.

Larval populations in Oneida Lake averaged 8.3 million in 1969, 1971, and 1973 when larvae were not stocked and 34.6 million in the 5 yr larvae were released (Table 1). Assuming the difference in numbers resulted from stocking, about 12% of the prolarvae released survived to the post-larval stage. The average interval between stocking and the population estimate was 10 days; thus the daily instantaneous mortality coefficient was 0.21. This mortality rate could not be sustained over a 40-day pelagic period without reducing the population to an unrealistically low number. To illustrate, the 1968 cohort would decline from 40.5 million to 9,000 which is less than the estimated population at age 4. Mortality during the transition from endogenous to exogenous feeding is either much higher than at later stages of development or stocked larvae experience much higher mortality than wild larvae.

Walleye larvae became concentrated in bays 10–15 days after hatching (Houde and Forney 1970) and localized aggregations hampered efforts to estimate mortality during the late pelagic period. Walleyes were captured in surveys designed to estimate abundance of yellow perch larvae (Noble 1968) 4–11 days after the initial estimate of the walleye population (Table 3). In most years the population showed little change between successive surveys which implied mortality was low although estimates were not sufficiently precise to provide quantitative assessment of mortality over a short time interval.

High variability in mortality during or immediately following the pelagic stage can be inferred from changes in ranking of year-classes between May and August (Table 2). Coefficients of variation for the eight year-classes increased between the larval and fingerling stages which indicated year-class size was diverging but no relation to initial density was evident. Judging from the ratio of fingerling caught in trawls to estimated larval population, survival was exceptionally low in 1973 and high in 1969. Since larval populations were low in both years it is obvious mortality from May to August was not strongly density-dependent.

Density-independent mortality during early stages of development has usually been attributed to inadequate food, to predation, or to interaction of these variables with climatic factors. Death from starvation seems improbable because number of zooplankters consumed by larval walleyes in 1961–63 was independent of zooplankton density over a wide range (Houde 1967). Predation is a more plausible explanation because larval walleyes were found in stomachs of yearling perch collected in tributaries and predation might continue in the lake while larvae are pelagic (Regier et al. 1969). However, comparison of yearling perch density estimated from catch in 20–30 trawl hauls in the spring (Forney

TABLE 4. Relative survival of walleye cohorts between the larval and fingerling stage and abundance of young and yearling yellow perch (*Perca flavescens*) from the catch in trawls.

Year	No. of walleye/ha		Relative survival May–Aug.	No. of yellow perch/ha	
	May[a]	Aug. 1[b]		May 1[b] (1)	Aug. 1[b] (0)
1966	1,580	35	0.0222	28	11,600
1967	1,060	93	0.0877	17	23,600
1968	1,960	216	0.1102	270[c]	34,800
1969	490	104	0.2122	578	7,000
1970	2,330	62	0.0266	2	13,500
1971	310	60	0.1935	153	45,700
1972	1,410	56	0.0397	50	2,400
1973	400	3	0.0075	4	1,200

Survival = 0.00571 + 0.000301 (age 1 perch) + 0.00000229 (age 0 perch)

[a]Population estimated when fry averaged 9–10 mm long.
[b]Based on c/f and area swept by trawls.
[c]Estimated from c/f of age 0 in October 1967 and mean c/f of age 1 in Aug.–Oct. 1968 assuming a constant exponential mortality since samples were not collected in May 1968.

1971) and relative survival of walleyes in 1966–73 failed to support the hypothesis (Table 4). Walleye survival was generally higher in years when yearling perch were abundant ($r = 0.77$).

Relative survival of walleye cohorts between May and August 1 was also correlated ($r = 0.53$) with abundance of young perch (Table 4). Young perch were captured in trawls during late July through October and the catch regressed over time to estimate relative size of perch year-classes on August 1 (Forney 1971). The correlation might be interpreted as evidence that factors that enhance survival of young walleyes also favor survival of young perch that hatch 10–15 days later. Heyerdahl and Smith (1971) suggested meteorological conditions during early development could explain parallel fluctuations in size of walleye and yellow perch year-classes recruited to the fishery in Red Lake, Minnesota. However, the positive correlation between walleye survival and abundance of both yearling and young perch in Oneida Lake suggests an alternative explanation.

Older walleyes feed on yearling perch in May and shift to young perch in June when the cohort attains a mean length of 18–20 mm (Forney 1974). Some young walleyes also appear in stomachs of older walleyes during June and July. Although frequency of occurrence of young walleyes in stomachs is low, density of young is also low and the number ingested may constitute a significant fraction of the year-class. High density of either or both yearling and young perch should reduce intensity of walleye cannibalism and enhance survival of walleye cohorts. As independent variables in a linear, multiple regression model, abundance of yearling and young perch accounted for much of the variability ($R^2 = 0.85$) in relative survival of young walleyes between May and August 1, 1966–73.

The smallest young walleye observed in the stomach of an older walleye was 20 mm and most predation occurred during the transition from the pelagic to demersal stage in late June or after young were demersal in July. The conclusion that cannibalism was responsible for variation in mortality between May and August 1 implies mortality during the early pelagic stage was low or stable. Since mortality of pelagic larvae was not measured, evidence supporting cannibalism is not entirely convincing. However, cannibalism has considerable impact on subsequent development of year-classes.

DEMERSAL STAGE

In an earlier paper Forney (1974) suggested mortality of young walleyes between August of the first and second summer of life was largely attributable to predation by older walleyes and intensity of predation was governed by density of the principal prey, young yellow perch. This conclusion was supported by three lines of evidence. First, number of young walleyes consumed by adults was a significant proportion of the fingerling population in 1969 when perch were scarce (Chevalier 1973). Second, electivity indices (Ivlev

1961) showed that selection for young walleyes by older walleyes increased as perch density decreased. And third, survival of the 1961–70 walleye year-classes between the first and second summer of life was closely correlated ($r = 0.86$) with abundance of young perch. Importance of forage fish abundance in modifying the impact of cannibalism is further illustrated by changes in abundance of young walleyes between August and October in 1966 through 1973.

Catches of young walleyes declined gradually in 1967, 1968, and 1971 and more rapidly in other years (Fig. 1). Coefficients of linear regressions fitted to log values of catches from July through October were compared with forage fish abundance. Weights of young perch and other forage-size fish taken in trawls were averaged over the period from July through October as an index of forage biomass. Young yellow perch comprised 50–95% of the biomass except in 1973 when young white perch (*Morone americanus*) predominated in the catch.

Mortality of fingerling walleyes as measured by regression coefficients was high in years when forage abundance was low (Fig. 2). Ivlev (1961) observed that predators consumed an increasing proportion of the available prey as prey density was reduced. Higher intensity of predation in years of low prey density is also consistent with observations that small year-classes of young yellow perch experienced higher mortality than numerically large year-classes during a period when predator biomass in Oneida Lake was stable (Forney 1971). With the exception of 1973, biomass of age 3 and older walleyes was between 20.3 and 30.5 kg/ha in 1966–73 (Table 5). Changes in prey selection probably contributed to the abrupt decrease in mortality of young walleyes at high forage fish densities. Electivity indices suggested young walleyes were selected by older walleyes in 1969 and 1970 when prey density was low and largely ignored in 1968 and 1971 when forage abundance was high (Forney 1974).

An alternative explanation for the relation shown in Fig. 2 is that fingerling walleyes starve in years when forage abundance is low. This seems improbable. Fingerlings can and frequently do subsist on a diet of invertebrates (Forney 1966).

Annual differences in forage abundance explain about 50% ($R^2 = .5055$) of the variability in mortality of fingerling walleyes if it is assumed that mortality decreased exponentially as prey abundance increased. Additional variables that could have affected intensity of predation in 1966–73 were predator density and growth of

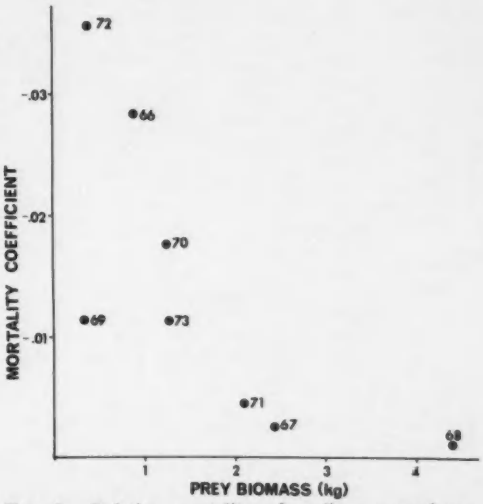

FIG. 2. Relative mortality of walleye year-classes determined from the decline in c/f and mean weight of forage-size fish taken in trawls in July through October 1966–73.

young walleyes. Biomass of age and older walleyes was low in 1973, but biomass of predators did not vary sufficiently over the 8-yr period to produce a discernible effect on mortality ($r = .02$). Inclusion of predator biomass as a second independent variable in a multiple regression with forage abundance did not appreciably reduce unexplained variation in fingerling mortality ($R^2 = .5231$).

Year-classes that grow rapidly may be less vulnerable to predation because Chevalier (1973) observed that adult walleyes selected smaller individuals in a cohort. Inclusion of total length of young walleyes on October 1 as a third independent variable results in little further reduction in variance ($R^2 = .5743$). However, a simple linear relation may not adequately express the relation between growth of a cohort and its vulnerability to predation. Length distributions of young walleyes are often skewed (Forney 1966) and mean length is not a precise measure of the proportion of the cohort vulnerable to predation. Differences in size composition of the adult walleye population and relative size of young walleyes and alternate prey could further obscure any relation between length of young walleyes and mortality during the summer. Because the number of potential variables is large, few conclusions could be drawn from analysis of available data.

Although it was not possible to demonstrate a relation between length of young walleyes and mortality rate during the first year of life, length

TABLE 5. Relative mortality of young walleyes between late July and mid-October, mean catch of forage-size fish in trawls, biomass of older walleyes, and mean total length of young walleyes on October 1 in 1966–73.

Year	Mortality coefficient[a]	Biomass of forage kg/haul	Biomass age 3 and older walleye kg/ha	Mean length (*mm*)
1966	−0.0283	0.88	24.6	142
1967	−0.0026	2.34	21.1	130
1968	−0.0013	4.42	20.8	145
1969	−0.0113	0.32	22.6	146
1970	−0.0178	1.24	21.3	136
1971	−0.0043	2.08	30.5	172
1972	−0.0358	0.36	20.3	127
1973	−0.0112	1.27	5.7	170

[a]Slope of regression fitted to trawl catches in Fig. 1.

attained by cohorts in the fall probably influenced subsequent survival. Young walleyes reached a mean length of 170–172 mm by October in 1971 and 1973 while young in other years averaged 127–146 mm (Table 5). Trawl catches suggest that an unusually high proportion of the young present in October of 1971 and 1973 survived to July–October of the following year (Table 2). Relative survival between the first and second year of life (c/f age 1 per c/f age 0 on October 15) was 0.84 and 0.82 for the 1971 and 1973 cohorts compared to 0.006 to 0.19 for other year-classes. High biomass of forage fish in the fall of 1971 and low biomass of older walleyes in 1973 (Table 5) probably reduced overwinter losses but these variables do not adequately explain the exceptionally high relative survival of the 1971 and 1973 year-classes. Possibly attainment of a threshold length affords young walleyes a high degree of protection from predation.

Discussion

Differences in size of walleye year-classes were not attributable to variability in early mortality. Losses during the egg and prolarval stage were high but remarkably stable. Comparison of egg production and the population of 8 mm larvae indicated a range in mortality of 0.9993–0.9995 in the 3 yr larvae were not stocked. Mortality in other years must have been very near these values otherwise variation in number of larvae originating from eggs spawned in the lake would have obscured the relation between abundance of larvae and number stocked. Low survival between the egg and post-larval stage in Oneida Lake is probably not unique. Faber (1967) captured few larval walleyes in two Wisconsin lakes and density in eight small New York lakes was similar to that observed in Oneida (Forney 1975b).

Changes in relative abundance of cohorts between May and August indicated considerable variation in annual mortality during the pelagic period or soon after young were demersal. Noble (1972) estimated abundance of a localized concentration of pelagic walleyes in a bay of Oneida Lake twice weekly in 1966 and 1967. The catch in high-speed samplers decreased rapidly as mean length of young increased from 10 to 18 mm over a 15-day period. After adjusting catches for avoidance of samplers the apparent mortality rate in both years was 0.95. However, the rate of decrease in catch of larvae in bays may not be a reliable measure of cohort mortality.

Year-classes in 1966 and 1967 apparently experienced lower larval mortality than the subpopulations studied by Noble. Density of larvae (9–10 mm) in May and fingerlings (60 mm) in August indicated a year-class mortality of about 0.98 in 1966 and 0.91 in 1967 during a 65–70 day period (Table 4). Actual survival from May to August may have been higher because trawl catches probably underestimate fingerling density (Chevalier 1973). Although losses between May and August were comparatively high in 1966 and 1967, about 20% of the post-larval walleyes survived to the fingerling stage in 1969 and 1971. Mean survival of the eight cohorts was 0.087 which is similar to survival of 0.078 reported for larvae stocked in natural rearing ponds in Wisconsin (Klingbiel 1971).

Cannibalism in late summer was responsible for much of the variability in year-class size although fingerling predation accounted for a small percentage of the total mortality experienced by a cohort. Intensity of cannibalism was keyed to abundance of young perch since few alternate prey were available (Forney 1974) and biomass of adult walleyes was fairly stable. Presumably similar fluctuations in recruitment could be in-

duced by varying density of older walleyes in a system where prey production was stable. Regier et al. (1969) speculated that overexploitation of blue pike (*Stizostedion vitreum glaucum*) in Lake Erie reduced predation on young blue pike and contributed to violent oscillations in recruitment.

Duration of cannibalism was determined by the growth rate of young walleyes. The 1971 and 1973 year-classes that attained a length of about 170 mm by mid-October experienced much lower mortality between fall and the following summer than cohorts that averaged 127–146 mm. The proportion of the adult walleye population capable of preying on walleyes over 170 mm is probably low. Parsons (1971) examined stomachs of walleyes ranging in length from 2 to 15.9 in. and plotted range in length of forage fish in stomachs against length of walleye. Maximum size of forage fish eaten increased steadily from 53 mm when walleyes were 114 mm long to 140 mm when walleyes averaged 394 mm long. Projecting the relation shown in fig. 1 of Parsons' paper, a 500-mm walleye is unlikely to consume prey exceeding 175 mm. Because only 16% of the female and virtually none of the male walleyes observed in spawning runs in Oneida Lake (Forney 1965a) exceed 500 mm, vulnerability of young walleyes to predation would decline sharply as they approached a length of 175 mm as in 1971 and 1973.

Cannibalism has not been reported as an important factor controlling recruitment in other walleye populations. This may reflect difficulties in detecting and assessing consumption of young. Density of young walleyes is frequently low even in lakes with well-established populations. Mraz (1968) found 9–19 fingerlings/ha in the fall and Kempinger and Churchill (1972) reported 37–220/ha in studies extending over several years in two Wisconsin lakes. Populations in both studies were estimated by mark and recapture. Density of young in Oneida Lake in October averaged about 50/ha judging from the catch and area swept by trawls. Consumption of 1–2 fingerlings during a season by each adult may have considerable impact on year-class size in lakes like Oneida where adult density usually exceeds 25/ha.

This study was not designed to evaluate walleye stocking but some comment on stocking seems unavoidable since initial size of walleye year-classes was strongly influenced by the release of hatchery reared larvae. These initial differences in year-class size did not persist and a comparison of number of larvae stocked and size of cohorts at age 1 would lead to the conclusion stocking was ineffective. However, the value of stocking should not be judged too harshly. A close relation between abundance of larvae and ultimate year-class size would not be anticipated in a population where first-year mortality varies by several magnitudes. Thus, any evaluation of stocking must rest on inferences drawn from the nature of the mortality process.

Much of the variation in size of walleye year-classes produced in Oneida Lake was attributable to predation by age 3 and older walleyes. Differences in abundance of young walleyes within the range observed in 1966–73 probably did not affect intensity of cannibalism. Since the number of predators capable of preying on a cohort was fixed by events in earlier years, the only variables likely to influence intensity of cannibalism were prey density and first-year growth of young walleyes. Biomass of young walleyes on August 1 never exceeded 1 kg/ha so as predators young walleyes were not sufficiently abundant to deplete the prey population and as prey, their contribution to the diet of older walleyes was negligible. Rapid growth of young in 1971 and 1973 when the larval population was not augmented could be interpreted as evidence of density-dependent growth, but factors other than density probably contributed to fast growth. Length of young walleyes at the end of the first growing season in 1956–64 varied directly with temperature and inversely with growth of young perch (Forney 1966). Thus, it seems likely that older walleyes would consume the same proportion of a year-class regardless of whether the cohort was augmented by stocking or not. The possibility that compensatory mortality is masked by more dramatic density-independent mortality cannot be rejected, but there is no a priori reason for assuming first-year mortality is compensatory.

Cannibalism in Oneida Lake can be viewed as a density-independent factor contributing to variation in year-class size and as a compensatory factor limiting population size. Bolstering the initial size of a cohort may increase the number of recruits but development of a strong year-class would suppress subsequent year-class through cannibalism. The value of supplemental stocking in an established population depends on success of natural reproduction, survival rate from factors other than cannibalism, and rate of exploitation of adult stock. Generalizations are impossible but it is clear that any general prejudice against larval stocking is unsound.

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Optimal Controls for a Single Species Fishery and the Economic Value of Research

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Mathematical proofs and analyses of solution methods are presented for determining optimal policies for the management of a single species fishery under equilibrium conditions. Previous intuitive arguments for solution of optimal policies controlling mesh size and fishing rate given complete information are explicitly proven. The analysis is extended to the case where some of the parameters describing the dynamics of the population are known only imprecisely to the manager. Using probability distributions for those unknown parameter values the problem is cast as a stochastic program where expected sustained net revenues from the fishery are maximized. The associated problem of optimal allocation of research resources under uncertainty conditions is considered by evaluating the direct value of such information to management activities.

Examples and algorithms are presented for the class of problems discussed.

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Nous présentons des preuves mathématiques et des analyses de méthodes de solution permettant de déterminer les politiques optimales pour la gestion d'une pêcherie d'une seule espèce dans des conditions d'équilibre. Les arguments antérieurs, de nature intuitive, sur lesquels étaient fondées les politiques optimales contrôlant la grandeur des mailles et le taux de pêche sont prouvés de façon explicite dans les cas où l'information est complète. Nous poursuivons plus loin l'analyse pour inclure les cas où certains paramètres de la dynamique de la population ne sont qu'imparfaitement connus de la gérance. La distribution des probabilités quant aux valeurs de ces paramètres permet de poser le problème sous forme de programme stochastique dans lequel les revenus nets anticipés de la pêcherie sont maximisés. Nous examinons le problème associé de l'allocation optimale des fonds pour la recherche dans des conditions d'incertitude en évaluant la valeur directe de telles connaissances pour la gestion.

Nous donnons des exemples et des algorismes applicables à la classe de problèmes examinés.

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THE purpose of this paper is to develop optimal policies for management of a single species fishery under equilibrium conditions. Two classes of models are developed and analyzed.

The first class consists of management models with perfect information. The objective function is assumed to be the maximization of net sustained revenue from the fishery. Optimal policies for controlling either mesh sizes or fishing rates are rigorously derived, establishing formally, apparently for the first time, the intuitive arguments of Beverton and Holt (1957). The results are then extended to the more complex case (characterizing long run policies) of simultaneously controlling mesh sizes and fishing rates.

The second class of models in this paper considers cases where some of the parameters that describe the dynamics of the fish population are not known precisely to the manager (e.g. natural mortality). The information concerning these parameters consists of probability distribution functions for "true" parameter values. The objective function is maximization of expected sustained net revenues from the fishery. An important associated problem in this case of management under

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uncertainty is the optimal allocation of resources to research activities. The procedures developed in this paper to evaluate the expected value of research activities consider only the direct value to management. Taking this approach, information does not have any intrinsic value, except the value it derives from its consequences of improved management (see for example Marschak 1971). The computational procedures are illustrated through application to Dickie and McCracken's data (1955) on *Pseudopleuronectes* stock in the St. Marys Bay, Nova Scotia.

The Beverton and Holt equilibrium yield model (Beverton 1953; Beverton and Holt 1956, 1957) is adopted to describe growth and mortality relationships in the managed fishery. In this model the number of fish of age t, $n(t)$ is given by (1) when no fishing takes place, and the number of recruits R is given by (2)

$$\frac{dn(t)}{dt} = -\mathrm{M}n(t) \text{ for } t_p \leqslant t \leqslant t_\lambda \tag{1}$$

$$\mathrm{R} = n(t_p) \tag{2}$$

Where M is the constant instantaneous rate of natural mortality, t_p is the age when the fish first enters the fishing grounds, and t_λ, the maximum age attained by the fish while vulnerable to fishing. The effects of fishing are reflected in $\mathrm{F}(t)$, the instantaneous rate of fishing mortality, which affects $n(t)$ simply by increasing mortality

$$\frac{dn(t)}{dt} = -[\mathrm{M} + \mathrm{F}(t)]n(t) \tag{3}$$

If it is assumed that all fish of ages greater than t_p are equally vulnerable to fishing, $\mathrm{F}(t)$ takes the form

$$\mathrm{F}(t) = \begin{cases} \mathrm{F} \text{ if } t_c \leqslant t \leqslant t_\lambda \\ 0 \text{ if } t < t_c \end{cases} \tag{4}$$

where the age of capture t_c is the average age at which the fish becomes vulnerable to the gear or mesh size under consideration.

The yield in number of fish, Y_n, is given by integrating $dn(t) = \mathrm{F}(t)n(t)dt$

$$\mathrm{Y}_n = \mathrm{F} \int_{t_c}^{t_\lambda} \mathrm{R}' \, \mathrm{e}^{-(\mathrm{F}+\mathrm{M})(t-t_c)} dt \tag{5}$$

where $\mathrm{R}' \equiv n(t_c)$

The yield in weight Y_w is given by

$$\mathrm{Y}_w = \mathrm{F} \int_{t_c}^{t_\lambda} \mathrm{R}'\mathrm{W}_t \, \mathrm{e}^{-(\mathrm{F}+\mathrm{M})(t-t_c)} dt \tag{6}$$

or

$$\mathrm{Y}_w = \mathrm{FRe}^{-\mathrm{M}(t_c-t_p)} \int_{t_c}^{t_\lambda} \mathrm{W}_t \, \mathrm{e}^{-(\mathrm{F}+\mathrm{M})(t-t_c)} dt$$

since $\mathrm{R}' = \mathrm{Re}^{-\mathrm{M}(t_c-t_p)}$

where W_t is the weight of the fish at age t.

It is assumed that the percentage increase in weight $(d\mathrm{W}_t/dt)/\mathrm{W}_t$ is a decreasing function of t. This assumption is valid for many growth curves (e.g. the Brody and the Bertalanffy curves).

Controls with Perfect Information

Single Parameter Optimization

First, optimal policies for controlling mesh size with a constant fishing rate will be explored. Similar arguments to the ones presented in this section were provided on an intuitive basis by

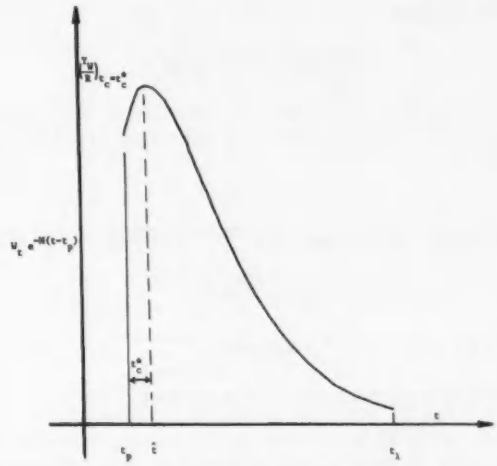

FIG. 1. Optimal yield in weight per recruit as a function of t_c^*.

Beverton and Holt (1957). In this case controls are imposed on the age of recruitment through choice of gear or through mesh size regulations. We assume that the demand for fish is infinitely elastic, i.e. there is a fixed price p per unit weight of the fish. Since C(F), the cost of fishing at a given fishing rate, and P are constants we can write the management problem simply as

$$\underset{t_c}{\text{Max}} \frac{Y_w}{R} = Fe^{-M(t_c - t_p)} \int_{t_c}^{t_\lambda} W_t e^{-(F+M)(t-t_c)} dt \tag{7}$$

$$\text{s.t.} \quad t_p \leqslant t_c \leqslant t_\lambda$$

In Appendix 1 we show that (Y_w/R) is a pseudoconcave function[1] of t_c over the range of fishable life span in the fishing grounds (t_p, t_λ); thus the global optimal point t_c^* is determined by the condition

$$\frac{\partial\left(\frac{Y_w}{R}\right)}{\partial t_c} = Fe^{-M(t_c - t_p)}\left[F \int_{t_c}^{t_\lambda} W_t e^{-(F+M)(t-t_c)} dt - W_{t_c}\right] = 0 \tag{8}$$

For $F \neq \infty$, this yields

$$W_{t_c^*} = F \int_{t_c^*}^{t_\lambda} W_t e^{-(F+M)(t-t_c^*)} dt \tag{9}$$

---

[1]In optimization many scientists invoke the assumption of concavity of the function to assert that a local maximum is also a global one. This assumption is stronger than necessary. The assumption of pseudoconcavity is sufficient. Consider the function $Y_w/R(t_c)$ (see Fig. 1). This function is not concave, but satisfies the conditions for pseudoconcavity. The main characteristic for a pseudoconcave function is that when it is increasing it will continue to increase until a maximum is reached; when it is decreasing it will never turn up.

Formally, pseudoconcavity is defined by the following condition:

$$\text{if} \quad \left(\frac{\partial f}{\partial x}\right)_{x=x_0}(x_1 - x_0) \leqslant 0 \quad \text{then} \quad f(x_1) \leqslant f(x_0).$$

Furthermore, it is clear that for a finite domain of the function say $[a, b]$, either $(\partial f/\partial x) = 0$ (if this exists) or max $[f(a), f(b)]$ will be the global maximum (Zangwill 1969).

For $F = \infty$, we have from (7) that

$$\frac{Y_w}{R} = e^{-M(t_c - t_p)} W_{t_c} \tag{10}$$

Hence $\dfrac{\partial\left(\dfrac{Y_w}{R}\right)}{\partial(t_c)} = 0$ yields

$$t_c^* = \hat{t} \tag{11}$$

Where the global optimal point $\hat{t}$ of biomass $W_t e^{-M(t-t_c)}$ is determined from the relation:

$$\dot{W}_{\hat{t}} \equiv \left.\frac{\partial W_t}{\partial t}\right|_{t=\hat{t}} = MW_{\hat{t}} \tag{12}$$

For certain F values, equation (8) does not have a solution. That is

$$\frac{\partial\left(\dfrac{Y_w}{R}\right)}{\partial t_c} > \text{or} < 0 \text{ for } t_p \leqslant t_c \leqslant t_\lambda \tag{13}$$

From Appendix 1 (see A1.8) it is known that

$$\frac{\partial\left(\dfrac{Y_w}{R}\right)}{\partial t_c} < 0 \text{ for } t_c > \hat{t}.$$

Thus we can only have the case

$$\frac{\partial\left(\dfrac{Y_w}{R}\right)}{\partial t_c} < 0 \text{ for } t_p \leqslant t_c \leqslant t_\lambda \tag{14}$$

Hence in this case we should set

$$t_c^* = t_p \tag{15}$$

From the results in the following section (29) we know that this can occur only for $F < F_p$ with F_p satisfying the following relation:

$$W_{t_p} = F_p \int_{t_p}^{t_\lambda} W_t e^{-(F_p + M)(t - t_p)} dt \tag{16}$$

(9), (11), and (15) constitute an optimal policy for controlling mesh size with a constant fishing rate.

To summarize: Given a fishing rate F, choose the age of recruitment (e.g. by selection of an appropriate mesh size) so as to satisfy the following:

$$t_c = t_p \text{ if } F < F_p \text{ with } W_{t_p} = F_p \int_{t_p}^{t_\lambda} W_t e^{-(F_p + M)(t - t_p)} dt$$

$$W_{t_c} = F \int_{t_c}^{t_\lambda} W_t e^{-(F + M)(t - t_c)} dt, \text{ if } F_p < F < \infty$$

$$t_c = \hat{t} \text{ with } \dot{W}_t = MW_{\hat{t}}, \text{ if } F = \infty$$

The value of t_c^* can be obtained by directly searching or plotting the left- and right-hand side function of (9) and locating the intersection point.

In Appendix 1 (A1.8) we established that

$$\frac{\partial\left(\frac{Y_w}{R}\right)}{t_c} < 0 \quad \text{for } t_c > \hat{t} \tag{17}$$

Thus the possibility that $t_c^* > \hat{t}$ is excluded. For any given fishing rate, the optimal value of t_c must be in the range $(t_p, \hat{t})$. That is, under any given fishing rate F, the t_c^* determined by the optimal policy, satisfies

$$t_p < t_c^* < \hat{t} \tag{18}$$

From (7), (9), (11), and (15) we get the optimal value

$$\left(\frac{Y_w}{R}\right)_{t_c=t_c^*} = W_{t_c^*} e^{-M(t_c^*-t_p)} \text{ for } F_p \leqslant F \tag{19}$$

This is the value of biomass $W_t e^{-M(t-t_p)}$ at the age t_c^*. Note that $(Y_w/R)_{t_c=t_c^*}$ a function of t_c^* has the same form as $W_t e^{-M(t-t_p)}$ a function of t (Fig. 1).

The second type of "single parameter" control arises when the age of recruitment is given by the current composition of gear and regulations on mesh sizes, but control of fishing rates is actively sought. Cost of fishing C(F) is taken to be an increasing function of the fishing rate. The control problem is stated as:

$$\underset{F}{\text{Max}} \left(p \cdot \frac{Y_w}{R} - \frac{C(F)}{R}\right) \tag{20}$$

$$\text{s.t. } F \geqslant 0$$

The problem of maximizing yield in weight is given as

$$\underset{F}{\text{Max}} \frac{Y_w}{R} = F e^{-M(t_c-t_p)} \int_{t_c}^{t_\lambda} W_t e^{-(F+M)(t-t_c)} dt \tag{21}$$

$$\text{s.t. } F \geqslant 0$$

Suppose F^* and $\hat{F}$ are the optimal solutions to problems (20) and (21), respectively. We note that

$$F^* \leqslant \hat{F} \tag{22}$$

as

$$p \cdot \frac{Y_w}{R}(F') - \frac{C(F')}{R} \leqslant p \cdot \frac{Y_w}{R}(\hat{F}) - \frac{C(F')}{R}$$

Since C(F) is an increasing function in F, it follows that

$$p \cdot \frac{Y_w}{R}(F') - \frac{C(F')}{R} \leqslant p \cdot \frac{Y_w}{R}(\hat{F}) - \frac{C(\hat{F})}{R}$$

$$\text{for all } F' \geqslant \hat{F}$$

In Appendix 2 we show that Y_w/R is a pseudoconcave function of F; thus, the global optimal solution for (21), $\hat{F}$, is determined by the condition

$$\frac{\partial\left(\frac{Y_w}{R}\right)}{\partial F} = e^{-M(t_c-t_p)}\left[\int_{t_c}^{t_\lambda} W_t e^{-(F+M)(t-t_c)} dt - F \int_{t_c}^{t_\lambda} W_t (t-t_c) e^{-(F+M)(t-t_c)} dt\right] = 0 \tag{23}$$

From (22), we know the optimal solution to problem (20) must be in the range $(0, \hat{F})$. Relation (A2.7) in Appendix 2 implies that $p \cdot Y_w/R$ is concave in this range. Assuming that $\partial C(F)/\partial F$ is an increasing function of F, the objective function in (20), $p \cdot Y_w/R - C(F)/R$ will be concave in F.

The global optimal solution to (20) will then be determined by the condition

$$p \cdot \frac{\partial\left(\frac{Y_w}{R}\right)}{\partial F} - \frac{\partial\left(\frac{C(F)}{R}\right)}{\partial F} = 0 \tag{24}$$

This states that optimality requires marginal revenue to equal marginal cost.

Simultaneous Control of Fishing Rates and Mesh Sizes

We have established in the previous section that for a given fishing rate F_0 there exists an optimal value of age of recruitment t_c^*. We now examine how $(Y_w/R)_{t_c=t_c^*}$ will be affected by a change in the fishing rate. Consider equation (9). The left-hand side of this condition of optimality is a constant $W_{t_c}^*$, while the right-hand side

$$F \int_{t_c^*}^{t_\lambda} W_t \, e^{-(F+M)(t-t_c^*)} dt$$

has the following properties:

1) it is equal to zero for $F = 0$
2) it intersects $W_{t_c}^*$ once only at $F = F_0$
3) it approaches $W_{t_c}^*$ when $F \to \infty$. These properties imply

$$\frac{\partial}{\partial F}\left(F \int_{t_c^*}^{t_\lambda} W_t \, e^{-(F+M)(t-t_c^*)} dt\right)\Bigg|_{F=F_0} \geqslant 0 \tag{25}$$

Since $(Y_w/R)_{t_c=t_c^*}$ is given by the multiplication of $e^{-M(t_c^*-t_p)}$, a term which does not contain F, with

$$F \int_{t_c^*}^{t_\lambda} W_t \, e^{-(F+M)(t-t_c^*)} dt,$$

it follows that

$$\frac{\partial\left(\frac{Y_w}{R}\right)_{t_c=t_c^*}}{\partial F}\Bigg|_{F=F_0} \geqslant 0 \tag{26}$$

This suggests with age of recruitment at optimum, a marginal increase in fishing rate from F_0 will lead to an increase in yield. However, a change in fishing rate may lead to changes in optimal ages of recruitment. Differentiating (9) with respect to F gives

$$\frac{\partial W_{t_c^*}}{\partial t_c^*}\frac{\partial t_c^*}{\partial F} = \frac{\partial}{\partial F}\left[F \int_{t_c^*}^{t_\lambda} W_t \, e^{-(F+M)(t-t_c^*)} dt\right] + MW_{t_c^*}\frac{\partial t_c^*}{\partial F}$$

or

$$[\dot{W}_{t_c^*} - MW_{t_c^*}]\frac{\partial t_c^*}{\partial F} = \frac{\partial}{\partial F}\left[F \int_{t_c^*}^{t_\lambda} W_t \, e^{-(F+M)(t-t_c^*)} dt\right] \tag{27}$$

From the last section we know that $\dot{W}_{t_c^*} - MW_{t_c^*} > 0$; thus,

$$\frac{\partial t_c^*}{\partial F}\Bigg|_{F=F_0} \geqslant 0 \tag{28}$$

This means that the optimal age of recruitment associated with a new fishing rate, $F > F_0$, will be larger than the one associated with F_0, and conversely for $F < F_0$ a smaller one.

From (28) we see that an increase in the fishing rate implies an increase in the optimal age of recruitment; in addition from (18) and (19) we see that an increase in optimal age of recruitment implies an increase in Y_w/R. Therefore the following relation is suggested:

$$\text{Increase in } F \Rightarrow \text{increase in } t_c^* \Rightarrow \text{increase } (Y_w/R) \tag{29}$$

(29) and (11) imply that the maximum sustained yield in weight (Y_w/R) is obtained for $F = \infty$ and $t_c^* = \hat{t}$. However, since by definition the marginal value of the fish at maximum yield level is zero, and marginal cost is positive; $F = \infty$ never maximizes net revenue.

To identify the long-run strategy satisfying the objective of maximization of net revenue, we have to solve the following problem

$$\underset{F, t_c}{\text{Max}}\ r = p \cdot Y_w - C(F) \tag{30}$$

$$\text{s.t. } t_p \leqslant t_c \leqslant t_\lambda$$

$$F > 0$$

For any F value, we have, from the analysis of single parameter policy, an optimal t_c value. Consequently we can modify (30) to take account of this correspondence, and reformulate our problem as follows:

$$\underset{F}{\text{Max}}\ r = p(Y_w)_{t_c = t_c^*} - C(F) \tag{31}$$

$$\text{s.t. } F > 0$$

where for any F value t_c^* is determined by the single parameter policy. From (A2.7) we know (25) implies

$$\frac{\partial^2}{\partial F^2}\left(F \int_{t_c^*}^{t_\lambda} W_t\, e^{-(F+M)(t-t_c^*)} dt\right)\Bigg|_{F=F_0} < 0 \tag{32}$$

(25) and (32) hold for all $F \geqslant F_p$ and its associated t_c^*. For $F < F_p$, we have $t_c^* = t_p$ (see (15)). Thus $(Y_w/R)_{t_c = t_c^*}$ takes the form

$$\left(\frac{Y_w}{R}\right)_{t_c = t_c^*} = F \int_{t_p}^{t_\lambda} W_t d^{-(F+M)(t-t_p)} dt \quad \text{for } F < F_p \tag{33}$$

Since

$$\frac{\partial\left(\frac{Y_w}{R}\right)_{t_c = t_c^*}}{\partial F}\Bigg|_{F=F_p} > 0,$$

the pseudoconcavity of (33) implies

$$\frac{\partial\left(\frac{Y_w}{R}\right)_{t_c = t_c^*}}{\partial F} > 0 \quad \text{for } F < F_p \tag{34}$$

Hence

$$\frac{\partial^2\left(\frac{Y_w}{R}\right)_{t_c = t_c^*}}{\partial F^2} < 0 \quad \text{for } F < F_p \tag{35}$$

(35) is derived from (A2.7).

(25), (34), (32), and (35) together indicate that $(Y_w/R)_{t_c = t_c^*}$ is an increasing, concave function of F. Thus, assuming that $\partial C(F)/\partial F$ is an increasing function of F, the net revenue r in (31) will be concave in F and the unique global optimal fishing rate F^* is determined by the condition:

$$\frac{\partial}{\partial F}[p \cdot (Y_w)_{t_c = t_c^*} - C(F)] = 0$$

or

$$p\left[\frac{\partial (Y_w)_{t_c = t_c^*}}{\partial F} + \frac{\partial (Y_w)_{t_c = t_c^*}}{\partial t_c^*}\frac{\partial t_c^*}{\partial F}\right] = \frac{\partial C(F)}{\partial F} \tag{36}$$

The first term in the bracket indicates the marginal revenue gained by a change in the fishing rate with the current optimal value t_c^*. The second term represents the marginal contribution to revenue of adjusting the value of t_c^* to the new fishing rate. The total equation provides the standard result in economics that optimality requires marginal revenue to equal marginal cost.

From (19) we get

$$\frac{\partial (Y_w)_{t_c = t_c^*}}{\partial F} = 0$$

$$\frac{\partial (Y_w)_{t_c = t_c^*}}{\partial_{t_c^*}} = [\dot{W}_{t_c^*} - MW_{t_c^*}]\, e^{-M(t_c^* - t_p)}$$

Using these and (27) we can write (36) as follows:

$$p \cdot e^{-M(t_c^* - t_p)} \frac{\partial}{\partial F}\left[F \int_{t_c^*}^{t_\lambda} W_t\, e^{-(F+M)(t - t_c^*)} dt\right] = \frac{\partial C(F)}{\partial F} \tag{37}$$

This provides the basic relation necessary to identify optimal fishing rate in conjunction with the conditions stipulated in the previous section for determination of optimal age of recruitment given a fishing rate. Appendices 3 and 4 provide modified standard algorithms for a single parameter and two parameter optimizations.

Optimal Management of a Single Species Fishery Under Uncertainty

In this section the management problem of a single fishery is modified to take account of uncertainties that exist with respect to the estimation of parameters determining the basic growth and mortality relationship in the system. Procedures to determine management decisions with respect to regulation of the fishery and allocation of research funds to reduce errors in parameters are described.

Optimization Under Uncertainty Conditions

The biological parameters that describe the basic relationship of the fishery are statistically derived estimates. A more accurate formulation of the fishery problem will describe these relationships in terms of probability distributions rather than point estimators. The management problem when no options for research and improved information are considered, can be cast as the following stochastic programming models.

For single parameter:

$$\max_{t_c} \underset{\underline{D}}{E} \frac{Y_w}{R}(\underline{D}, t_c) \qquad \max_{F} \underset{\underline{D}}{E}\, r(\underline{D}, F)$$

$$\text{s.t. } F \geqslant 0$$

$$\text{s.t. } t_c(\underline{D}) = t_p(\underline{D}) \text{ if } t_c < t_p(\underline{D})$$

$$t_c(\underline{D}) = t_\lambda(\underline{D}) \text{ if } t_c > t_\lambda(\underline{D}) \tag{38}$$

For simultaneous optimization

$$\begin{aligned} &\underset{t_c,F}{\text{Max}}\ \underset{\underline{D}}{\text{E}}\ r(\underline{D}, t_c, F) \\ &\text{s.t. } t_c(\underline{D}) = t_p(\underline{D}) \text{ if } t_c < t_p(\underline{D}) \\ &\quad t_c(\underline{D}) = t_\lambda(\underline{D}) \quad t_c > t_\lambda(\underline{D}) \\ &\quad F \geqslant 0 \end{aligned} \tag{39}$$

where the vector $\underline{D}$ represents all the stochastic parameters.

Research activities to improve estimates of parameters can be described in terms of costs of research and the new probability distribution expected as a result of this research for determination of management policies. For example, a research proposal may indicate the research budget and the expected reduction of variation (say reduction in the standard deviation for a normal distribution of error). The economic value of new research depends on the extent the information provided by it leads to improvements in management practice resulting in additional revenues.

Suppose the initial information concerning a parameter is given by the distribution G_0. Proposed research will result in one of the distributions $G_1(\underline{D}')$. All the parameters of this distribution are specified in the proposal except of course $\underline{D}'$, say the mean of the distribution that can be obtained only after the research is concluded. The stochastic variable $\underline{D}'$ has the prior distribution G_0.

The economic value of this research proposal can be assessed by computing the expected net gain in revenue resulting from this research.

Formally, $V(G_0, G_1)$, the economic value of the proposed research, can be written as

$$V(G_0, G_1) = \underset{\underline{D}'}{\text{E}}\ \underset{t_c,F}{\text{Max}}\ \underset{\underline{D}^+}{\text{E}}\ r(\underline{D}^+, t_c, F) - \underset{t_c,F}{\text{Max}}\ \underset{\underline{D}'}{\text{E}}\ \underset{\underline{D}^+}{\text{E}}\ r(\underline{D}^+, t_c, F) \tag{40}$$

subject to the constraints

$$\begin{aligned} &t_c(\underline{D}^+) = t_p(\underline{D}^+) \text{ if } t_c < t_p(\underline{D}^+) \\ &t_c(\underline{D}^+) = t_\lambda(\underline{D}^+) \text{ if } t_c > t_\lambda(\underline{D}^+) \\ &F \geqslant 0 \end{aligned}$$

where the stochastic variable $\underline{D}^+$ represents the true value.

In the first term of (40)

$$\underset{t_c,F}{\text{Max}}\ \underset{\underline{D}^+}{\text{E}}\ r(\underline{D}^+, t_c, F)$$

represents the expected revenue given the particular mean $\underline{D}'$. Thus stochastic variable $\underline{D}^+$ is described by the distribution function $G_1(\underline{D}')$. To obtain the expected revenue prior to the research, one must weigh the alternative possible outcomes of the research by their respective probabilities of occurrence. These are given by the distribution G_0.

The second term represents the expected revenue when management is based solely on prior information.

(40) can be written as

$$V(G_0, G_1) = \underset{\underline{D}'}{\text{E}}\left[\underset{t_c,F}{\text{Max}}\ \underset{\underline{D}^+}{\text{E}}\ r(\underline{D}^+, t_c, F) - \underset{\underline{D}^+}{\text{E}}\ r(\underline{D}^+, t_c^0, F^0)\right] \tag{41}$$

with t_c^0, F^0 denoting the values that optimize

$$\underset{\underline{D}'}{\text{E}}\ \underset{\underline{D}^+}{\text{E}}\ r(\underline{D}^+, t_c, F).$$

The bracket in (41) is always positive, so we get

$$V(G_0, G_1) \geqslant 0 \tag{42}$$

The value of perfect information is a special case of (41), where G_1 is a Dirac delta function or equivalently

$$\underset{\underset{\sim}{D}^+}{E}\ r(\underset{\sim}{D}^+, t_c, F) = r(\underset{\sim}{D}^+, t_c, F).$$

An optimal policy concerning allocation to research effort that improves estimates of a parameter for a given fishing season should equate the cost of the research $C(G_1)$ with $V(G_0, G_1)$ the value of the information provided.

Solution Method

Calculations of the solution of the stochastic program, $\text{Max}_x E_t f(x, t)$ and the distribution problem, $E_t \text{Max}_x f(x, t)$ are generally quite formidable. Solution methods for the distribution problem are particularly difficult as one must generally solve $\text{Max}_x f(x, t)$ for all t considered. However when f is convex (or concave) in t, a simple computational procedure generating sharper and sharper bounds, can be applied.[2] By convexity, we have the Jensen's and Madansky's inequality (Madansky 1959; Huang and Ziemba 1974)

$$f(\mu^0) \leqslant \underset{t}{E} f(t) \leqslant \frac{b - \mu^0}{b - a} f(a) + \frac{\mu^0 - a}{b - a} f(b) \tag{43}$$

with

$$\mu^0 = \int_a^b t dF(t) \quad \text{and} \quad \int_a^b dF(t) = 1$$

where $F(t)$ is the distribution function of t. Subdividing (a, b) into (a, μ^0) (μ^0, b) and applying both inequalities on each of these subintervals we have

$$C_1{}^1 f(\mu_1{}^1) \leqslant \underset{t\in(a,\mu^0)}{E} f(t) \leqslant C_1{}^1 \left[\frac{\mu^0 - \mu_1{}^1}{\mu^0 - a} f(a) + \frac{\mu_1{}^1 - a}{\mu^0 - a} f(\mu^0) \right] \tag{44}$$

$$C_2{}^1 f(\mu_2{}^1) \leqslant \underset{t\in(\mu^0,b)}{E} f(t) \leqslant C_2{}^1 \left[\frac{b - \mu_2{}^1}{b - \mu^0} f(\mu^0) + \frac{\mu_2{}^1 - \mu^0}{b - \mu^0} f(b) \right] \tag{45}$$

with

$$C_1{}^1 = \int_a^{\mu^0} dF(t); \quad C_2{}^1 = \int_{\mu^0}^b dF(t)$$

$$\mu_1{}^1 = \int_a^{\mu^0} t dF(t)/C_1{}^1; \quad \mu_2{}^1 = \int_{\mu^0}^b t dF(t)/C_2{}^1$$

Recently Huang and Ziemba (1974) have shown that

$$f(\mu^0) \leqslant C_1{}^1 f(\mu_1{}^1) + C_2{}^1 f(\mu_2{}^1) \leqslant \underset{t}{E} f(t) \leqslant C_1{}^1 \left[\frac{\mu^0 - \mu_1{}^1}{\mu^0 - a} f(a) + \frac{\mu_1{}^1 - a}{\mu^0 - a} f(\mu^0) \right]$$
$$+ C_2{}^1 \left[\frac{b - \mu_2{}^1}{b - \mu^0} f(\mu^0) + \frac{\mu_2{}^1 - \mu^0}{b - \mu^0} f(b) \right] \leqslant \frac{b - \mu^0}{b - a} f(a) + \frac{\mu^0 - a}{b - a} f(b) \tag{46}$$

Clearly, the subintervals (a, μ^0) (μ^0, b) can be subdivided further and yield the sharper bounds. Consequently by choosing a suitable n, such that the lower bound is approximately equal to the upper bound

$$\sum_{i=1}^{2^n} C_i{}^n f(\mu_i{}^n) \cong \sum_{i=1}^{2^n} C_i{}^n \left[\frac{\bar{c}_i{}^n - \mu_i{}^n}{\bar{c}_i{}^n - \underline{c}_i{}^n} f(\underline{c}_i{}^n) + \frac{\mu_i{}^n - \underline{c}_i{}^n}{\bar{c}_i{}^n - \underline{c}_i{}^n} f(\bar{c}_i{}^n) \right] \tag{47}$$

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[2]For example Y_w/R is convex in M for any fixed value of t_c, F.

one can approximate $E_t f(t)$ by

$$\underset{t}{E} f(t) = \sum_{i=1}^{2^n} C_i^n f(\mu_i^n) = \sum_{i=1}^{2^n} C_i^n \left[\frac{\bar{c}_i^n - \mu_i^n}{\bar{c}_i^n - \underline{c}_i^n} f(\underline{c}_i^n) + \frac{\mu_i^n - \underline{c}_i^n}{\bar{c}_i^n - \underline{c}_i^n} f(\bar{c}_i^n) \right] \tag{48}$$

with

$$C_1^1 = \int_a^{\mu^0} dF(t), C_2^1 = \int_{\mu^0}^b dF(t); \quad \mu_1^1 = \int_a^{\mu^0} t dF(t)/C_1^1, \mu_2^1 = \int_{\mu^0}^b t dF(t)/C_2^1 \tag{49}$$

$$C_i^K = \int_{\underline{c}_i^K}^{\bar{c}_i^K} dF(t); \quad \mu_i^K = \int_{\underline{c}_i^K}^{\bar{c}_i^K} t dF(t)/C_i^K$$

$$C_{2i-1}^{K+1} = \int_{\underline{c}_i^K}^{\mu_i^K} dF(t), C_{2i}^{K+1} = \int_{\mu_i^K}^{\bar{c}_i^K} dF(t); \quad \mu_{2i-1}^{K+1} = \int_{\underline{c}_i^K}^{\mu_i^K} t dF(t)/C_{2i-1}^{K+1},$$

$$\mu_{2i}^{K+1} = \int_{\mu_i^K}^{\underline{c}_i^K} t dF(t)/C_{2i}^{K+1}$$

Therefore $\operatorname{Max}_x E_t f(x, t)$ and $E_t \operatorname{Max}_x f(x, t)$[3] can be simplified as

$$\operatorname*{Max}_x \sum_{i=1}^{2^n} C_i^n f(x, \mu_i^n) \quad \text{and} \quad \sum_{i=1}^{2^n} C_i^n \operatorname*{Max}_x f(x, \mu_i^n),$$

respectively. And so we need only to solve a finite number of optimization problems.

Numerical Examples

We have chosen as an example to consider management of *Pseudopleuronectes*. Vital statistics and Bertalanffy growth parameters for this stock in the St. Marys Bay, Nova Scotia were published by Dickie and McCracken (1955) as follows:

age of fish entering the fishing ground	$t_p = 3$ yr
maximum age of exit from fishing	$t_\lambda = 18$ yr
natural mortality rate	$M = 0.25$
weight curve $W_t = W_\infty(1 - e^{-k(t-t_0)})^3$	
with the following parameters	$k = 0.4$, $W_\infty = 2.43$ lb and $t_0 = 0$

We shall apply the bounding method to examine the optimal policy when there is uncertainty in M and research on M is proposed. We consider only the case of mesh size optimization with a given fishing rate, as the computational procedures with variable F are similar. We assume that the distribution function G_0, which provides the prior information with respect to M, is uniform with the range (0.2, 0.3).

The optimal policy problem under uncertainty without research options can be stated formally as:

$$\operatorname*{Max}_{t_c} \underset{M}{E} \frac{Y_w}{R}(M, t_c).$$

$$\text{s.t. } t_p \leqslant t_c \leqslant t_\lambda \tag{50}$$

[3] $\operatorname{Max}_x f(x, t)$ is convex in t if $f(x, t)$ is convex in t for any fixed value of x.

For $n = 0$, we have, from (47), the lower and upper bounds of (50)

$$\underset{t_c}{\text{Max}}\ C_1{}^0 \frac{Y_w}{R}(\mu_1{}^0, t_c) \tag{51}$$

$$\underset{t_c}{\text{Max}}\ C_1{}^0 \left[\frac{\bar{c}_1{}^0 - \mu_1{}^0}{\bar{c}_1{}^0 - \underline{c}_1{}^0}\frac{Y_w}{R}(\underline{c}_1{}^0, t_c) + \frac{\mu_1{}^0 - \underline{c}_1{}^0}{\bar{c}_1{}^0 - \underline{c}_1{}^0}\frac{Y_w}{R}(\bar{c}_1{}^0, t_c)\right] \tag{52}$$

$$\text{s.t. } t_p \leqslant t_c \leqslant t$$

with

$$C_1{}^0 = \int_a^b dF(t) = \int_{0.2}^{0.3} 10\,dt = 1,\ \mu_1{}^0 = \mu^0 = \int_a^b t dF(t) = \int_{0.2}^{0.3} 10\,t dt = 0.25$$

$$\underline{c}_1{}^0 = a = 0.2,\ \bar{c}_1{}^0 = b = 0.3$$

The optimal values of the deterministic nonlinear programming problems (51) and (52) are 0.916 and 0.921, respectively, with the associated $t_c{}^*$ 3.495 and 3.524.

For $n = 1$, the lower and upper bounds of (50) are given by the optimal values of (53) and (54), respectively.

$$\underset{t_c}{\text{Max}}\left[C_1{}^1 \frac{Y_w}{R}(\mu_1{}^1, t_c) + C_2{}^1 \frac{Y_w}{R}(\mu_2{}^1, t_c)\right] \tag{53}$$

$$\underset{t_c}{\text{Max}}\left[C_1{}^1\left(\frac{\bar{c}_1{}^1 - \mu_1{}^1}{\bar{c}_1{}^1 - \underline{c}_1{}^1}\frac{Y_w}{R}(\underline{c}_1{}^1, t_c) + \frac{\mu_1{}^1 - \underline{c}_1{}^1}{\bar{c}_1{}^1 - \underline{c}_1{}^1}\frac{Y_w}{R}(\bar{c}_1{}^1, t_c) + C_2{}^1\left(\frac{\bar{c}_2{}^1 - \mu_2{}^1}{\bar{c}_2{}^1 - \underline{c}_2{}^1}\frac{Y_w}{R}(\underline{c}_2{}^1, t_c) + \frac{\mu_2{}^1 - \underline{c}_2{}^1}{\bar{c}_2{}^1 - \underline{c}_2{}^1}\frac{Y_w}{R}(\bar{c}_2{}^1, t_c)\right)\right)\right] \tag{54}$$

$$\text{s.t. } t_p \leqslant t_c \leqslant t$$

with

$$C_1{}^1 = \int_a^{\mu_0} dF(t) = \int_{0.2}^{0.25} 10\,dt = 0.5,\ C_2{}^1 = \int_{\mu_0}^b dF(t) = \int_{0.25}^{0.3} 10\,dt = 0.5$$

$$\mu_1{}^1 = \int_a^{\mu_0} t dF(t)/C_1{}^1 = \int_{0.2}^{0.25} 10\,t dt/0.5 = 0.225$$

$$\mu_2{}^1 = \int_{\mu_0}^b t dF(t)/C_2{}^1 = \int_{0.25}^{0.3} 10\,t dt/0.5 = 0.275$$

$$\underline{c}_1{}^1 = \underline{c}_1{}^0 = 0.2,\ \bar{c}_1{}^1 = \mu_1{}^0 = 0.25$$

$$\underline{c}_2{}^1 = \mu_1{}^0 = 0.25,\ \bar{c}_2{}^1 = \bar{c}_1{}^0 = 0.3$$

Thus, the lower and upper bounds are 0.917 and 0.919, respectively, with the associated $t_c{}^*$ values of 3.502 and 3.509.

Similarly, for $n = 2$ we have the bounds (0.918, 0.918) and the associate $t_c{}^*$ values (3.504, 3.504). $t_c{}^* = 3.504$ yr if a precision up to 10^{-3} is desired (clearly such precision is impractical in real management situations and is provided here for pedagogical reason only).

$$\underset{t_c}{\text{Max}}\ \underset{M}{E}\frac{Y_w}{R}(M, t_c) = 0.918 \text{ lb}$$

Now assume that a research project is proposed to provide "perfect information" on M (practically reduces error to a point to which computational procedures are insensitive). It is desirable to compute the maximal amount management should be willing to allocate to conduct such research.

Clearly if the costs of such research exceed the value of improvements in management due to the information it provides, the research project should be rejected.

Formally the value of perfect information is given by:

$$V(G_0, G_1 = \text{Delta function}) = \underset{M'}{E}\ \underset{t_c}{\text{Max}}\ Y_w(M', t_c) - \underset{t_c}{\text{Max}}\ \underset{M'}{E}\ Y_w(M', t_c)$$

The second term has already been calculated. The first term has the bounds (see (51) and (52))

$$\left(\underset{t_c}{\text{Max}}\ Y_w(0.25, t_c), \frac{1}{2}\underset{t_c}{\text{Max}}\ Y_w(0.2, t_c) + \frac{1}{2}\underset{t_c}{\text{Max}}\ Y_w(0.3, t_c)\right) = (0.916, 0.929) \quad \text{for } n = 0$$

$$\left(\frac{1}{2}\underset{t_c}{\text{Max}}\ Y_w(0.225, t_c) + \frac{1}{2}\underset{t_c}{\text{Max}}\ Y_w(0.275, t_c), \frac{1}{4}\underset{t_c}{\text{Max}}\ Y_w(0.2, t_c)\right.$$

$$\left. + \frac{1}{2}\underset{t_c}{\text{Max}}\ Y_w(0.25, t_c) + \frac{1}{4}\underset{t_c}{\text{Max}}\ Y_w(0.3, t_c)\right) \quad \text{(see (53) and (54)).}$$

$$= (0.920, 0.923) \quad \text{for } n = 1$$

and similarly

$$(0.921, 0.921) \quad \text{for } n = 2$$

If this degree of accuracy is sufficient the computation ends with

$$\underset{M'}{E}\ \underset{t_c}{\text{Max}}\ Y_w(M', t_c) = 0.921 \quad \text{in units of R.P (recruits} \times \text{price)}$$

And

$$V(G_0, G_1(M) = \text{delta function})$$

$$= 0.921 - 0.918$$

$$= 0.03 \quad \text{in units of R.P.}$$

If instead of perfect information a research proposal suggests obtaining a more favorable distribution for M, say $G_1(M)$, then its value can be stated as

$$V(G_0, G(M)) = \underset{M'}{E}\ \underset{t_c}{\text{Max}}\ \underset{M^+}{E}\ Y_w(M^+, t_c) - \underset{t_c}{\text{Max}}\ \underset{M'}{E}\ \underset{M^+}{E}\ Y_w(M^+, t_c)$$

$E_{M^+}Y_w(M^+, t_c)$ is convex in M' since summation of convex functions is a convex function. Also $\text{Max}_{t_c} E_{M^+}Y_w(M^+, t_c)$ is convex in M' since $E_{M^+}Y_w(M^+, t_c)$ is convex in M' for all t_c values. Thus, the bounding techniques applied in the example of perfect information can be applied here to evaluate both $E_{M'}$ and E_{M^+}.

Appendix 1

In this section we shall prove that Y_w/R is a pseudoconcave function of t_c over the range of fishable life span (t_p, t_λ).

We have

$$\frac{Y_w}{R} = F\, e^{-M(t_c - t_p)} \int_{t_c}^{t_\lambda} W_t\, e^{-(F+M)(t - t_c)} dt \tag{A1.1}$$

Partial differentiation with respect to t_c yields:

$$\frac{\partial\left(\frac{Y_w}{R}\right)}{\partial t_c} = F\, e^{-M(t_c - t_o)}\left[F \int_{t_c}^{t_\lambda} W_t\, e^{-(F+M)(t - t_c)} dt - W_{t_c}\right] \tag{A1.2}$$

and

$$\frac{\partial^2\left(\frac{Y_w}{R}\right)}{\partial t_c^{\,2}} = F\,e^{-M(t_c-t_p)}\left\{F\left[F\int_{t_c}^{t_\lambda} W_t\,e^{-(F+M)(t-t_c)}dt - W_{t_c}\right] + [MW_{t_c} - \dot{W}_{t_c}]\right\} \tag{A1.3}$$

where

$\dot{W}_{t_c} \equiv \partial W_{t_c}/\partial t_c$. Set t_c^* such that

$$\left.\frac{\partial\left(\frac{Y_w}{R}\right)}{\partial t_c}\right|_{t_c=t_c^*} = 0 \tag{A1.4}$$

From (A1.2) and (A1.3), we than have, for $F \neq \infty$

$$\left.\frac{\partial^2\left(\frac{Y_w}{R}\right)}{\partial t_c^{\,2}}\right|_{t_c=t_c^*} = F\,e^{-M(t_c^*-t_p)}[MW_{t_c^*} - \dot{W}_{t_c^*}] \tag{A1.5}$$

$$<0 \text{ if } t_c^* < \hat{t}$$

and

$$>0 \text{ if } t_c^* > \hat{t} \tag{A1.6}$$

where $\hat{t}$ is defined as

$$\dot{W}_{\hat{t}} = MW_{\hat{t}} \tag{A1.7}$$

$\hat{t}$ is the global maximum point of the pseudoconcave function $W_t\,e^{-M(t-t_p)}$, since $\dot{W}_t/W_t$ is decreasing in t.

The fact that the biomass $W_t\,e^{-M(t-t_p)}$ is a pseudoconcave function implies that $W_t\,e^{-M(t-t_p)}$ is a decreasing function over $(\hat{t}, t_\lambda)$. This in turn implies

$$\frac{\partial\left(\frac{Y_w}{R}\right)}{\partial t_c} < 0 \quad \text{for } t_c > \hat{t} \tag{A1.8}$$

Therefore t_c^* lies in the range $(t_p, \hat{t})$. From (A1.6) we know that $(Y_w/R)(t_c)$ is a pseudoconcave over $(t_p, \hat{t})$. This gives

$$\frac{\partial\left(\frac{Y_w}{R}\right)}{\partial t_c} > 0 \quad \text{for } t_c < t_c^* \tag{A1.9}$$

$$\frac{\partial\left(\frac{Y_w}{R}\right)}{\partial t_c} < 0 \quad \text{for } t_c^* < t_c \leqslant \hat{t} \tag{A1.10}$$

(A1.8), (A1.9), and (A1.10) show that for $F \neq \propto Y_w/R$ is pseudoconcave in t_c over (t_p, t_λ).

For $F = \infty$

$$\frac{Y_w}{R} = W_{t_c}\,e^{-M(t_c-t_p)} \tag{A1.11}$$

This has the same form as the biomass function.

Thus for $F = \infty$, Y_w/R is pseudoconcave in t_c over (t_p, t_λ).

Appendix 2

In this section we shall prove that Y_w/R is a pseudoconcave function of F.

We have

$$\frac{Y_w}{R} = e^{-M(t_c - t_p)} F \int_{t_c}^{t_\lambda} W_t e^{-(F+M)(t-t_c)} dt = e^{-M(t_c - t_p)} f(F) \tag{A2.1}$$

With

$$f(F) \equiv F \int_{t_c}^{t_\lambda} W_t e^{-(F+M)(t-t_c)} dt \tag{A2.2}$$

Partial differentiation with respect to F yields

$$\frac{\partial f(F)}{\partial F} = \int_{t_c}^{t_\lambda} W_t e^{-(F+M)(t-t_c)} dt - F \int_{t_c}^{t_\lambda} W_t (t - t_c) e^{-(F+M)(t-t_c)} dt$$

$$= e^{-(F+M)(t_\lambda - t_c)} W_{t_\lambda} (t_\lambda - t_c) - \int_{t_c}^{t_\lambda} (\dot{W}_t - MW_t)(t - t_c) e^{-(F+M)(t-t_c)} dt \tag{A2.3}$$

(A2.3) is obtained from the following relation

$$\int_{t_c}^{t_\lambda} W_t (t - t_c) e^{-(F+M)(t-t_c)} dt = -\frac{1}{F+M} e^{-(F+M)(t-t_c)} W_t (t - t_c) \Big|_{t_c}^{t_\lambda}$$

$$+ \frac{1}{F+M} \int_{t_c}^{t_\lambda} W_t e^{-(F+M)(t-t_c)} dt + \frac{1}{F+M} \int_{t_c}^{t_\lambda} \dot{W}_t (t - t_c) e^{-(F+M)(t-t_c)} dt \tag{A2.4}$$

Similarly

$$\frac{\partial^2 f(F)}{\partial F^2} = -2 \int_{t_c}^{t_\lambda} W_t (t - t_c) e^{-(F+M)(t-t_c)} dt + F \int_{t_c}^{t_\lambda} W_t (t - t_c)^2 e^{-(F+M)(t-t_c)} dt$$

$$= -\left[e^{-(F+M)(t_\lambda - t_c)} W_{t_\lambda} (t_\lambda - t_c)^2 - \int_{t_c}^{t_\lambda} (\dot{W}_t - MW_t)(t - t_c)^2 e^{-(F+M)(t-t_c)} dt \right] \tag{A2.5}$$

For $\frac{\partial f(F)}{\partial F} \geqslant 0$, we have from (A2.3)

$$e^{-(F+M)(t_\lambda - t_c)} W_{t_\lambda} (t_\lambda - t_c)^2 \geqslant \int_{t_c}^{t_\lambda} (\dot{W}_t - MW_t) \frac{(t_\lambda - t_c)}{(t - t_c)} (t - t_c)^2 e^{-(F+M)(t-t_c)} dt$$

$$> \int_{t_c}^{t_\lambda} (\dot{W}_t - MW_t)(t - t_c)^2 e^{-(F+M)(t-t_c)} dt \tag{A2.6}$$

i.e.

$$\frac{\partial^2 f(F)}{\partial F^2} < 0 \quad \text{for} \quad \frac{\partial f(F)}{\partial F} \geqslant 0 \tag{A2.7}$$

(A2.7) shows that $f(F)$ is pseudoconcave in F. (A2.1) implies that Y_w/R is a pseudoconcave function of F.

Appendix 3

Below is an algorithm for Modified Fibonacci Search to determine the optimal t_c and Y_w/R for a given F

1. Set TMIN $= t_p$
   TMAX $= \hat{t}$
   SDI = smallest distinguishable interval
2. Determine the Fibonacci numbers FIB and FIBL set FIBL < (TMAX − TMIN)/SDI ⩽ FIB
3. If TMAX − TMIN < SDI go to step 5. Otherwise generating the Fibonacci number

$$\text{FIBH} = \text{FIB}$$

$$\text{FIB} = \text{FIBL}$$

$$\text{FIBL} = \text{FIBH} - \text{FIBL}$$

and set $t_c = \text{TMIN} + \text{FIB*SDI}$

4. Calculate $\dfrac{\partial\left(\dfrac{Y_w}{R}\right)}{\partial t_c} = F\int_{t_c}^{t_\lambda} W_t\, e^{-(F+M)(t-t_c)}dt - W_{t_c}$ and

$$\left(\frac{Y_w}{R}\right) = e^{-M(t_c-t_p)}F\int_{t_c}^{t_\lambda} W_t\, e^{-(F+M)(t-t_c)}dt$$

If $\dfrac{\partial\left(\dfrac{Y_w}{R}\right)}{\partial t_c} = 0$ go to step 6.

If $\dfrac{\partial\left(\dfrac{Y_w}{R}\right)}{\partial t_c} > 0$ set TMIN = TC

$$\left(\frac{Y_w}{R}\right)_+ = \left(\frac{Y_w}{R}\right)$$

otherwise set TMAX = TC

$$\left(\frac{Y_w}{R}\right)_- = \frac{Y_w}{R}$$ go to step 3

5. If $\left(\dfrac{Y_w}{R}\right)_+ > \left(\dfrac{Y_w}{R}\right)_-$ choose TC = TMIN and $\dfrac{Y_w}{R} = \left(\dfrac{Y_w}{R}\right)_+$

otherwise TC = TMAX and $\dfrac{Y_w}{R} = \left(\dfrac{Y_w}{R}\right)_-$

6. Finish

Appendix 4

This appendix presents an algorithm of complex direct search routine for solving

$$\max_{F,t_c} r = p\cdot Y_w - C(F)$$

$$\text{s.t. } t_p \leqslant t_c \leqslant \hat{t}$$

$$F > 0$$

1. Find an initial feasible solution t_c^F, F^F
2. Generate a simplex

$$t_c^1 = t_c^F, \quad t_c^2 = t_c^1 + \delta_1, \quad t_c^3 = t_c^1$$

$$F^1 = F^F, \quad F^2 = F^1, \quad F^3 = F^1 + \delta_2$$

That is, find δ_1, δ_2 such that t_c^2 and F^3 are feasible

3. Determine the worst vector, namely (t_c^W, F^W) where

$$r(t_c^W, F^W) = \underset{1 \leqslant i \leqslant 3}{\text{MIN}}\ r(t_c^i, F^i)$$

break ties arbitrarily. Determine the centroid of the remaining vectors

$$t_c^c = \sum_{i \neq W} t_c^i/2,\ F^c = \sum_{i \neq W} F^i/2$$

stopping rule: δ is specified
Stop if the r value at the centroid changes by less than δ in the last five iterations, that is

$$\underset{1 \leqslant \alpha, \beta \leqslant 5}{\text{Max}} \left| r(t_c^c(\alpha), F^c(\alpha)) - r(t_c^c(\beta), F^c(\beta)) \right| < \delta$$

4. Over reflect away from (t_c^W, F^W) through (t_c^c, F^c) to the trial point

$$t_c^T = t_c^c + \alpha(t_c^c - t_c^W),\ F^T = F^c + \alpha(F^c - F^W) \cdot \alpha > 1$$

5. If $t_p \leqslant t_c^T \leqslant \hat{t}$, $F^T > 0$ and $r(t_c^T, F^T) > r(t_c^W, F^W)$ replace the worst point by the trial point and go to 3.

6. If the trial point is infeasible, return to step 5 with a trial point $(t_c^{T'}, F^{T'})$ that moves an amount 10^{-6} inside any violated constraint

7. If the trial point is feasible but $r(t_c^T, F^T) \leqslant r(t_c^W, F^W)$ move halfway toward the centroid, that is

Let $t_c^H = \dfrac{t_c^W + T_c^C}{2}$, $F^H = \dfrac{F^W + F^C}{2}$.

If (t_c^H, F^H) is feasible, go to 3 with (t_c^H, F^H) replacing (t_c^W, F^W).

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Effects of Low Concentrations of Oil Accommodated in Sea Water on the Growth of Unialgal Marine Phytoplankton Cultures[1]

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Effects of three oils accommodated in sea water upon the growth of unialgal cultures of marine phytoplankton were investigated. The oil concentrations used covered the range normally encountered in sea water subjected to oil pollution (<1 mg/liter). In most experiments, minor stimulation or inhibition of growth was apparent but in only two experiments was the growth of oil-contaminated cultures statistically different from controls (in both instances stimulation). Extrapolation of the results to the natural environment is complicated by several factors, the most important of which appear to be variability in organism response and the observation that oil concentration and composition change during experiments. Oil concentrations encountered in polluted sea water can affect the growth of phytoplankton, but the effects seem to be minor and short-lived.

PROUSE, N. J., D. C. GORDON JR., AND P. D. KEIZER. 1976. Effects of low concentrations of oil accommodated in sea water on the growth of unialgal marine phytoplankton cultures. J. Fish. Res. Board Can. 33: 810–818.

Nous avons étudié les effets de trois concentrations d'huile incorporées dans l'eau de mer sur la croissance de cultures unialgales de phytoplancton marin. Les concentrations utilisées se situent dans l'intervalle normalement rencontré dans l'eau de mer polluée par l'huile (<1 mg/litre). Dans la plupart des essais, une faible stimulation ou une inhibition de la croissance est apparente, mais la croissance des cultures contaminées par l'huile n'est statistiquement différente des témoins que dans deux essais (une stimulation dans les deux cas). L'extrapolation des résultats à l'environnement naturel est compliquée à cause de plusieurs facteurs, dont les plus importants semblent être la variabilité dans la réponse des organismes et le fait que la concentration et la composition de l'huile changent durant les expériences. Les concentrations d'huile trouvées dans l'eau de mer polluée peuvent affecter la croissance du phytoplancton, mais les effets semblent être mineurs et de courte durée.

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BECAUSE a measurable portion of petroleum hydrocarbons entering the aquatic environment find their way into the water column, investigation of the environmental impact of oil pollution must include studies of possible effects on plankton. In the past few years numerous studies involving marine and freshwater phytoplankton have been reported (Gordon and Prouse 1973; Kauss et al. 1973; Mommaerts-Billiet 1973; Nuzzi 1973; Shields et al. 1973; Lacaze 1974; Pulich et al. 1974; and Soto et al. 1975a, b). The present state of knowledge in this rapidly expanding area of research has been reviewed recently by Vandermeulen and Ahern (1976). Firm general conclusions are impossible to make at this time due to the wide variety of oils, phytoplankton species, and experimental conditions that have been used. Another difficulty is accurately extrapolating the results of laboratory experiments to conditions encountered in the natural environment.

Our research in this field has centered on studying the possible effects of aqueous oil concentrations less than 1 mg/liter on the photosynthesis and growth of marine phytoplankton. This range includes those concentrations generally encountered in the marine environment, under

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both natural and polluted conditions. Our initial work (Gordon and Prouse 1973) examined the short-term (1 day) effects of three representative oils on the radiocarbon uptake of natural phytoplankton populations from Bedford Basin, N.S., and the northwest Atlantic Ocean between Halifax and Bermuda. The results suggested that oil concentrations as low as about 50 μg/liter could reduce the photosynthetic potential of phytoplankton. Concentrations of this level and higher can be encountered in the immediate vicinity of oil spills and in chronically polluted coastal waters.

In this paper, we present the results of recent investigations on the effects of oil accommodated in sea water on the growth of laboratory cultures of phytoplankton species common to Nova Scotian coastal waters. These experiments were of longer term, lasting an average of 11 days and extending through at least two generations of cells. Careful attention was paid to following the changes in hydrocarbon composition and concentrations in seawater growth media during experiments.

Methods

Oils Investigated

Three oils were investigated: Venezuelan crude, Kuwait crude, and No. 2 fuel. The first was obtained from the Imperial Oil Refinery in Dartmouth, N.S. and was used in our previous experiments (Gordon and Prouse 1973). The latter two oils are from the American Petroleum Institute (API) reference collection and were also used by Pulich et al. (1974).

Preparation of Oil-Contaminated Sea Water

Filtered sea water (0.45-μ Millipore filter), collected at 30 m in St. Margaret's Bay and having a background hydrocarbon concentration of about 1 μg/liter, and whole oil were autoclaved separately at 15 lb/in^2 for 15 min. The oil (25 ml) was contained in a 50-ml Erlenmeyer flask stoppered with a foam plug and covered by several layers of paper held in place with an elastic band. As discussed below, some volatile hydrocarbons were undoubtedly lost. After autoclaving, an unmeasured amount (10–50 mg) of oil was added to 300 ml of filtered sea water and shaken vigorously by hand in a sterilized glass separatory funnel for 5 min. After sitting undisturbed for 30 min, oil-contaminated sea water (having an oil concentration of about 50 mg/liter) was drawn from the bottom of the separatory funnel and used immediately in experiments. Because oil concentrations in culture media were measured at the beginning and end of each experiment, it was not necessary to know the exact amount of oil initially added to sea water.

Chemical Analysis of Hydrocarbons

Fluorescence spectroscopy — The contents of a culture flask (100 ml) were extracted with 10 ml of either pentane or hexane (redistilled from glass) in a glass separatory funnel. The extract was analyzed directly using the techniques of Gordon and Keizer (1974). Fluorescence detects aromatic hydrocarbons, which are more water soluble than the aliphatics, but our experience indicates that reasonably good estimates of total oil concentrations can be obtained by using the oil dissolved in solvent as a standard. Qualitative information on aromatic hydrocarbons was obtained in Experiments 6 and 10 by running synchronous excitation of emission spectra.

Gas chromatography — The contents of culture flasks (100 ml) were extracted twice with 100 ml of pentane (redistilled twice from glass). The combined extract was washed with 50 ml saturated aqueous sodium chloride solution, dried over anhydrous sodium sulfate, concentrated to 5 ml in a Kuderna–Danish evaporator, and finally concentrated to 0.5 ml under a stream of purified nitrogen. The concentrated extract was eluted with 3.5 ml pentane from a prerinsed column (0.4 cm ID $\times$ 10 cm) containing 4 cm silica gel (60–200 mesh, grade 950, activated at 250 C for 16 h) under 1-cm alumina (Brockmann activity grade 1, activated for 16 h at 400 C). The eluate was concentrated to 100 μl under nitrogen.

Samples were analyzed on a Perkin–Elmer 3920 gas chromatograph equipped with an MS-41 sampling accessory on a 12 ft $\times$ $\frac{1}{8}$ in OD stainless steel column packed with 2.2% Apeizon L on 60/80 mesh Chromosorb W (AW) (programmed from 70 to 270 C at 8 C/min with a flow of 30 cm^3 helium/min) and on a Perkin–Elmer 990 gas chromatograph equipped with an inlet splitter on a 50 ft $\times$ 0.02 in ID OV-101 SCOT column (programmed from 90 to 270 C at 6 C/min). Identification was based on retention times compared with standards.

Algal Growth Experiments

Growth experiments were conducted with five species of phytoplankton randomly chosen from a collection of cultures isolated from Nova Scotian coastal waters by Dr S. R. Durvasula of our laboratory in 1967: *Dunaliella tertiolucta*, *Fragilaria* sp., *Monochrysis* sp., *Skeletonema* sp., and *Chaetoceros* sp. These algae were cultured using axenic techniques at 10 C under constant light (Sylvania Lifeline natural white fluorescent bulbs, 30 mW/m^2) in F/2 medium (modified Guillard) made up with filtered sea water collected at 30 m in St. Margaret's Bay.

Algae were cultured in 300-ml Erlenmeyer flasks containing 100 ml of medium. Each experiment consisted of a control and three to five different oil concentrations, each in triplicate. Oil-contaminated sea water was not added to cultures until they had entered the exponential growth phase. At this stage, cultures with cell concentrations deviating more than 10% from the mean were discarded to insure that

all flasks had similar cell concentrations. The volume of oil-contaminated sea water added ranged between 0.1 and 4 ml (1 ml produced an initial concentration of about 500–1000 μg/liter in the medium). Culture flasks were stoppered with foam plugs and sealed with brown paper and elastic bands. Each flask was swirled daily to mix contents and returned to a different position in the growth chamber to remove any bias due to illumination or temperature gradients within the growth chamber.

Algal growth was measured approximately every second day by withdrawing 1 ml of culture with a sterile pipette, diluting it in 200 ml sea water that had been filtered through a 0.45-μ Millipore filter, and determining cell volume (ppm) and cell number with a Model T Coulter Counter using a 100-μ aperture. Counts of oil-contaminated sea water indicated that under the conditions employed oil particles were not having a significant effect on cell volume and number determinations. The 10 experiments conducted continued for 9–16 days after oil-contaminated sea water was added to cultures.

Additional culture flasks (inoculated with algae in Experiments 1–3 but not thereafter) were prepared and the contents extracted at the beginning and end of each experiment for hydrocarbon analysis.

Results

Differences in Composition Between Fresh and Autoclaved Kuwait Crude Oil

There were no pronounced measurable differences in composition between fresh and autoclaved Kuwait crude oil. The synchronous excitation of emission spectra (SEES) (Gordon and Keizer 1974) of aromatic compounds and the relative concentrations of n-alkanes were not affected by autoclaving oil. There could have been a substantial loss of aliphatic hydrocarbons with boiling points less than n-decane (bp 174) and of aromatic hydrocarbons such as benzene and alkylbenzenes during autoclaving. These more volatile hydrocarbons have extremely short half-lives in sea water, on the order of minutes (Mackay and Wolkoff 1973) and therefore will not persist long in the natural environment. It thus appears that autoclaving oil in bulk is acceptable for experiments such as ours which are designed to examine the effects of prolonged exposure of phytoplankton to oil accommodated in sea water.

Composition of Kuwait Crude Oil Accommodated in Sea Water

The composition of Kuwait crude oil accommodated in sea water is different from the parent oil. Differences were greater at the end of a 12-day experiment than at the beginning. The SEES at the beginning (30 min) was not appreciably

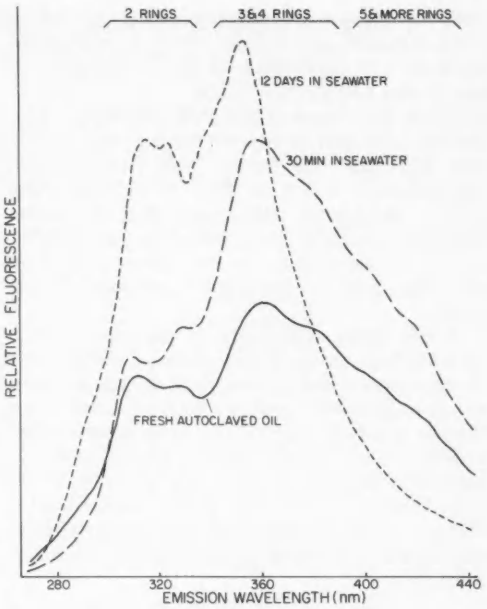

FIG. 1. Synchronous excitation of emission spectra (SEES) of Kuwait crude oil: 1) freshly autoclaved, 2) accommodated in sea water after 30 min, and 3) accommodated in sea water after 12 days. The general types of aromatic compounds producing the fluorescence in each region are identified.

different from the parent oil (Fig. 1). Any loss of low boiling aromatics, such as naphthalene (bp 217), by evaporation was apparently compensated for by its greater solubility. The composition of n-alkanes at 30 min, however, was markedly different from the parent oil (Fig. 2). The relative concentrations of n-alkanes below n-octadecane (C18, bp 308) were reduced by evaporation. n-Decane and n-undecane were lost completely. After 12 days, the SEES had changed substantially and indicated a persistence of 2- to 4-ring aromatics (Fig. 1). At the same time, most of the n-alkanes below n-eicosane (C20, bp 330) had disappeared (Fig. 2) so that the most abundant n-alkanes were C20–C22. The composition of n-alkanes after 12 days was different in those culture flasks inoculated with algae (*Dunaliella* and *Chaetocetos*). The anomalous peak at C18 probably represents phytane produced by the algae which was not completely resolved from n-octadecane on the chromatographic column used. The relative concentrations of n-alkanes between n-nonadecane (C19) and n-pentacosane (C26) were reduced in the presence of algae suggesting utilization of some kind.

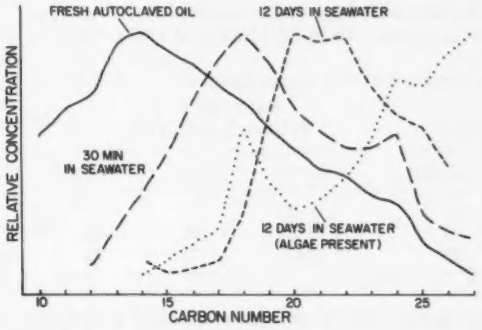

FIG. 2. Relative concentrations of n-alkanes in Kuwait crude oil: 1) freshly autoclaved, 2) accommodated in sea water after 30 min, 3) accommodated in sea water after 12 days, and 4) accommodated in sea water after 12 days in the presence of algae.

Changes in the composition of oil accommodated in sea water are also reflected in the C17: pristane ratio (Table 1). There was preferential disappearance of the n-alkane relative to the isoprenoid in culture flasks both with and without algae. This disparity cannot be explained by evaporation, but suggests that cultures were not completely sterile and that n-alkanes were being degraded by bacteria during experiments.

These results demonstrate that the composition of hydrocarbons accommodated in sea water and available to algae during these experiments is markedly different from the parent oil and changes constantly with time. At the end of the experiments the predominant hydrocarbons remaining in media are those that are least volatile, most soluble, and presumably most resistant to biological alteration (i.e. medium molecular weight aromatics).

Changes in Oil Concentration During a 12-Day Experiment

In addition to the changes in composition illustrated above, oil concentrations also change during experiments. In the presence of algae, concentrations of both total oil (as estimated by fluorescence spectroscopy) and n-alkanes (as determined by GLC) in sea water contained in culture flasks dropped 92–94% in 12 days (Table 2). Similar decreases were detected in the growth experiments (Tables 3 and 4). The fact that algae are exposed to constantly changing oil concentrations (generally decreasing) complicates the interpretation of growth experiments.

TABLE 1. Changes in the n-C17:pristane ratio observed in Experiments 6 and 10. Chromatograms produced on an OV-101 SCOT column.

Sample	n-C17:pristane
1. Freshly autoclaved	5.30
2. Accommodated after 30 min	4.27
3. Accommodated after 12 days, without algae	1.20
4. Accommodated after 12 days, with algae	0.9–1.25

Growth of Algae

Initially, the growth data were plotted and examined. Most observations were made while the algae were growing at their maximum rates (i.e. exponential growth phase). The last few observations in Experiments 7, 8, and 9 were made after the cultures had reached the plateau stage. Typical growth curves are presented in Fig. 3 and 4. On the basis of the growth curves, experiments were divided into two groups; those that suggested no or a stimulatory effect of oil on growth (Table 3) and those that suggested an inhibitory effect (Table 4).

Next, linear regressions of replicate sets (control and each oil concentration) were calculated using all data (the log of cell volume was used in Experiments 7–9 which plateaued) and the slopes within each experiment were compared by covariance analysis (Li 1957). In two experiments, the F values indicated that the slope of at least one replicate set was significantly greater than the slope of the control at the 95% level (Table 3). Thus, an initial concentration of 50 μg/liter of No. 2 fuel oil stimulated the growth of *Fragilaria* and initial concentrations of 55 and 106 μg/liter of Kuwait crude oil stimulated the growth of *Dunaliella* (Fig. 4). In all other instances, the apparent stimulation and inhibition was not statistically significant (Tables 3 and 4). Qualitative observations did indicate that inhibition was more pronounced at higher oil concentrations and stimulation at lower concentrations.

Finally, the data were examined qualitatively to see whether or not the growth of oil-contaminated cultures varied in relation to controls during experiments as the oil concentration and composition changed. Changes were evident but quite variable (Tables 3 and 4). In Experiment 5 (Fig. 3), No. 2 fuel oil appeared to inhibit the growth of *Dunaliella* the first week after oil was added but thereafter the growth of oil-contaminated cultures caught up to and in some instances surpassed that of controls. Therefore, no significant differences were detected when all data were tested (Table 4).

TABLE 2. Changes in hydrocarbon concentrations observed in seawater media contained for 12 days in culture flasks inoculated with algae. Total n-alkanes include C10 to C27. Estimated total hydrocarbons based on fluorescence analysis.

	Total n-alkanes ($\mu g/liter$)			Estimated total hydrocarbons ($\mu g/liter$)		
Seawater samples	Initial	Final	% Loss	Initial	Final	% Loss
Control (n=2)	1	2	—	2	6	—
A Replicates (n=2)	110	9	92	2950	174	94
B Replicates (n=2)	46	3	93	1050	73	93

TABLE 3. Experiments showing no effect or an apparent stimulatory effect of oil on phytoplankton growth. KC = Kuwait crude, VC = Venezuelan crude, and No. 2 = No. 2 fuel oil. ND = oil concentrations not determined. Experiment number indicates sequence.

			Oil concn ($\mu g/liter$)			
Exp. no.	Oil	Alga	Initial	Final	F value	Qualitative observations
2	VC	*Dunaliella*	54–285	ND	1.0	General slight stimulation
3	No. 2	*Fragilaria*	50–230	ND	10.7[a]	Stimulation at all concentrations increasing with time
4	KC	*Fragilaria*	110–790	8–70	0.1	Stimulation during days 0–5 at lower concentrations
6	KC	*Dunaliella*	55–320	12–38	6.0[a]	Stimulation at all stages which decreased with increasing concentrations
7	KC	*Fragilaria*	43–950	12–180	0.1	General slight stimulation
9	KC	*Skeletonema*	350–5000	67–1250	0.5	No apparent difference

[a] $P > 95\%$.

TABLE 4. Experiments showing an apparent inhibitory effect of oil on phytoplankton growth. VC = Venezuelan Crude, KC = Kuwait crude, and No. 2 = No. 2 fuel oil. ND = oil concentrations not determined. Experiment number indicates the sequence.

			Oil concn ($\mu g/liter$)			
Exp. no.	Oil	Alga	Initial	Final	F Value	Qualitative observations
1	VC	*Dunaliella*	19–66	ND	3.8	Inhibition at higher concn increasing with time
5	No. 2	*Dunaliella*	50–380	2–45	2.0	Inhibition decreasing with time
8	KC	*Monochrysis*	174–1800	22–315	1.1	Inhibition increasing with time and oil concn
10	KC	*Chaetoceros*	270–5000	—	0.6	Inhibition during days 6–10

The generation times (the time required for the number of cells to double) ranged between 4 and 6 days. Each experiment, therefore, extended through at least two and in some cases four generations of algae. There were no detectable differences in cell diameter with time in any of the 10 experiments.

Discussion

Our experiments did not show any clear differences in the relative toxicity of the three oils. No. 2 fuel oil has frequently been reported to be the oil most toxic to phytoplankton (Gordon and Prouse 1973; Nuzzi 1973; and Pulich et al. 1974). There also were no consistent differences in the response of different phytoplankton species. Because the growth of *Dunaliella* and *Fragilaria* was significantly stimulated in two instances, these species may be more tolerant to oil than the others tested. Other investigations (Kauss et al. 1973; Nuzzi 1973; and Pulich et al. 1974) have demonstrated that phytoplankton species can be quite variable in their sensitivity to oil, an ob-

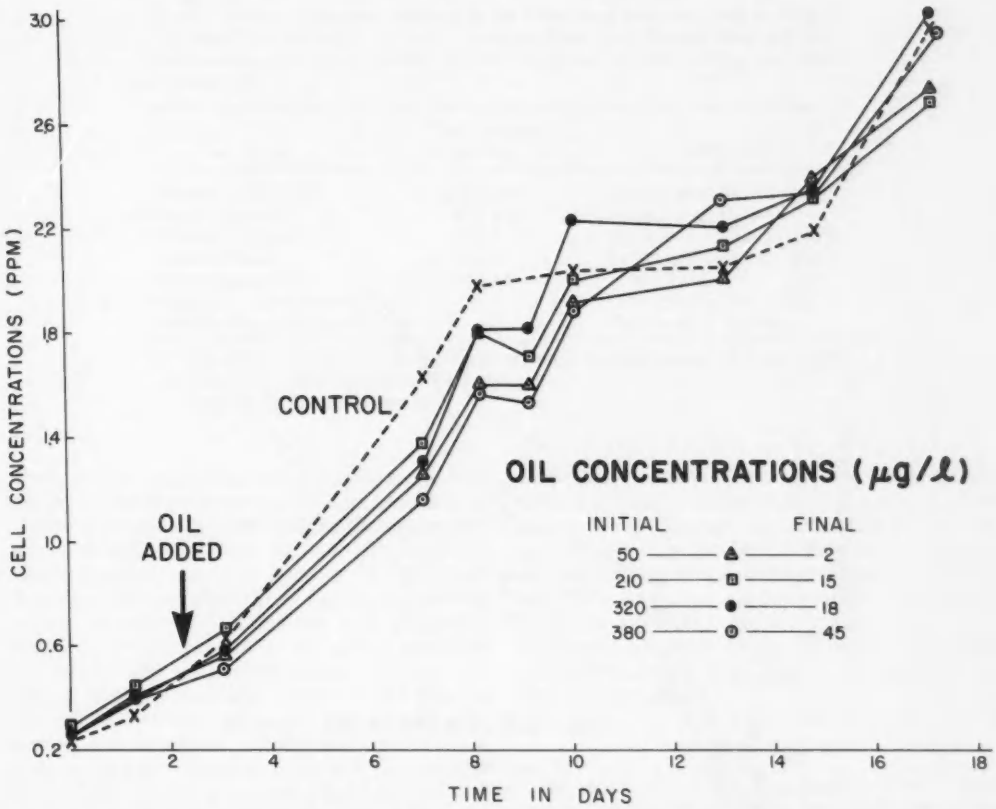

FIG. 3. Growth data from Experiment 5; *Dunaliella* and No. 2 fuel oil. Each point represents the mean cell concentration in three replicate culture flasks.

servation that suggests the possibility of oil pollution affecting the species composition of natural phytoplankton populations.

Our experiments were conducted after cultures had left the lag phase and while they were growing at their maximum rate. It has recently been demonstrated (Mommaerts-Billiet 1973; Pulich et al. 1974; and Soto et al. 1975) that exposure to oil can prolong the lag phase. Pulich et al. (1974) present evidence that this can occur at concentrations lower than those that affect growth.

The interpretation of results is complicated by the realization that the composition and concentration of petroleum hydrocarbons present in the culture media change continually during experiments. The longer the experiments, the greater the changes in the oil and its potential toxicity. The composition and concentration of oil accommodated in sea water in the natural environment also changes constantly due to weathering. It is therefore impossible to ascribe a measured effect to a specific hydrocarbon or concentration. Nevertheless, we believe that using the concentration ranges observed in experiments, general conclusions can be reached which provide insight into the possibility of oil affecting the growth of phytoplankton in eastern Canadian marine waters.

As in our earlier work (Gordon and Prouse 1973), stimulation was most pronounced at the lowest oil concentrations (initially 50–100 µg/liter) (Table 3). Contrary to our previous studies, we did not observe any strong inhibition. However, suggestions of minor inhibitions were evident (Table 4). This variance in results could be explained by the duration of the experiments (1 vs. an average of 11 days) and perhaps by differences in analytical methods.

The results of this and other investigations make it clear that the growth of phytoplankton present in sea water receiving initial oil concentrations of 1 mg/liter or less can be substantially

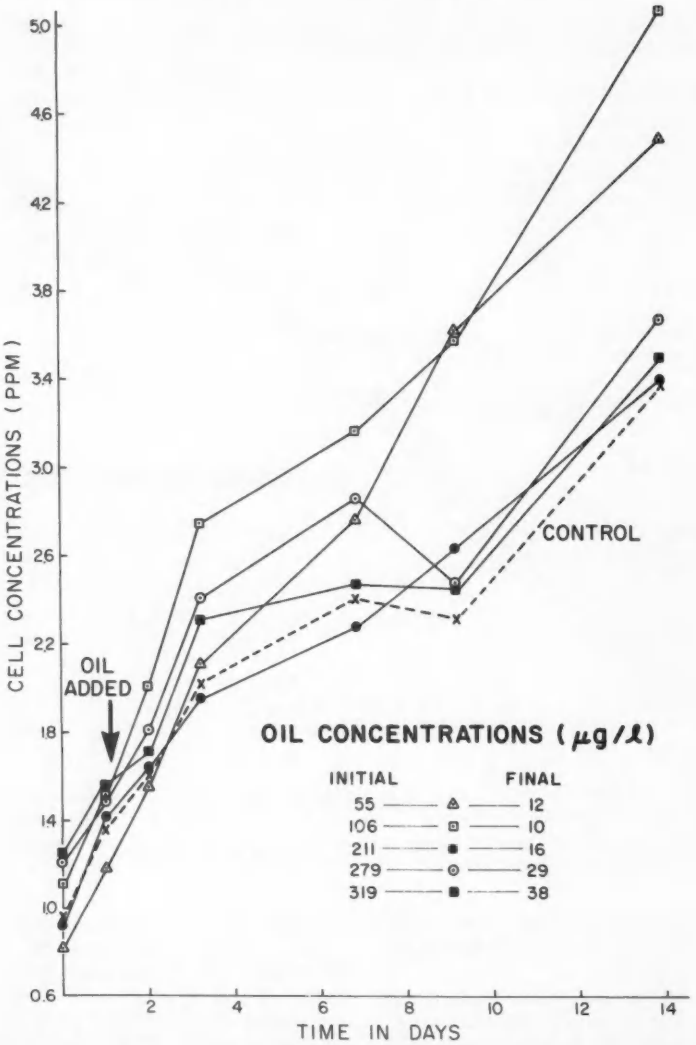

FIG. 4. Growth data from Experiment 6; *Dunaliella* and Kuwait crude oil. Each point represents the mean cell concentration in three replicate culture flasks. The stimulation apparent at the initial concentrations of 55 and 106 μg/liter is statistically significant.

affected. If the concentrations are very low (less than about 100 μg/liter stimulation can occur. Inhibition can occur at higher concentrations although it may be shortlived due to the relatively short half-life of oil in the water column. Concentrations in this range are consistently observed in waters subjected to substantial oil spills and chronic oil pollution (Table 5). Therefore, concern of the possible effects of oil on important ecological processes such as primary production by marine phytoplankton in such situations is, still, very much warranted. Much remains to be learned about what the exact effects are and how they will affect the phytoplankton community over the long term.

The importance of experimental details in any studies dealing with the sublethal effects of petroleum hydrocarbons cannot be overemphasized.

TABLE 5. Oil concentrations reported in sea water from areas subjected to different degrees of oil pollution. All concentrations represent estimates obtained using fluorescence spectroscopy except those of Ahmed et al. (1974) which were determined by IR.

Area	Concn range (*μg/liter*)	Reference
Simulated oil spill[a]	2600–21	Gordon et al. (1975)
Boston Harbour	816–114	Ahmed et al. (1974)
Halifax Harbour	72–2	Michalik and Gordon (1971)
Bedford Basin	60–1	Keizer and Gordon (1973)
Chedabucto Bay[b]	41–16	Levy (1971)
Gulf of St. Lawrence	10–1	Levy and Walton (1973)
Halifax–Bermuda Section	<1	Gordon et al. (1974)

[a]One liter of oil added to 8000 liters of sea water in outdoor tank and oil concentrations in sea water monitored for 3 mo.
[b]Three months after *Arrow* spill.

Such factors as autoclaving oil, methods for preparing oil-contaminated water, and duration of experiments all affect the hydrocarbon chemistry of test solutions. When oil and sea water are mixed together, individual hydrocarbons can react in many different ways. They can evaporate, dissolve, complex with dissolved and particulate material already present in sea water, and form emulsions. The relative importance of these different reactions depends largely on the kind of oil, the ratio of oil to sea water, type of container, means and duration of mixing, and other factors. Our experiments demonstrate that the composition of hydrocarbons accommodated in sea water is different than in the parent oil and that this difference increases with time (Table 1, Fig. 1 and 2). Most of the oil initially accommodated in raw sea water is particulate in nature (Gordon et al. 1973; and Boehm and Quinn 1974) and therefore filtering test solutions before experiments, as practiced by Pulich et al. 1974), has a pronounced effect on hydrocarbon concentration and composition and produces conditions not normally encountered in the natural environment.

All experimental problems must be thoroughly appreciated by everyone studying the biological ramifications of petroleum hydrocarbons in sea water if meaningful data are to be obtained. These problems can be overcome by using standard oils and procedures, conducting an adequate number of replicate experiments to correct for the expected variability in response, and insuring that hydrocarbon analyses of test solutions are performed while experiments are in progress. Equal emphasis must be placed on the biological and chemical aspects of the problem.

Our experiments provide additional evidence that the levels of petroleum hydrocarbons attainable in sea water subjected to heavy oil pollution can be high enough to affect phytoplankton growth but that the effects may be minor and short-lived. The details of phytoplankton response and their long-term implications are not clearly understood. Further research in this area is justified and our experiments illustrate additional procedural details that should be included in future experiments.

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Diel Ecdysis Rhythms in Juvenile Lobsters *Homarus americanus*

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TAMM, G. R., AND J. S. COBB. 1976. Diel ecdysis rhythms in juvenile lobsters *Homarus americanus*. J. Fish. Res. Board Can. 33: 819–821.

Juvenile lobsters, held both individually and in pairs, molt primarily during daylight. Eighty-four and 81.4% of the animals molting into the fifth and sixth stages, respectively, did so between 0600 and 1800. The presence of shelter did not significantly affect this pattern, nor did five different holding conditions.

TAMM, G. R., AND J. S. COBB. 1976. Diel ecdysis rhythms in juvenile lobsters *Homarus americanus*. J. Fish. Res. Board Can. 33: 819–821.

Des jeunes homards, gardés individuellement ou en paires, muent surtout le jour. Parmi le individus passant aux cinquième et sixième stades, 84 et 81.4% respectivement muent entre 0600 et 1800 h. La présence d'un abri n'affecte pas ce rythme de façon significative, non plus que cinq conditions différentes de maintien en vivier.

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ALTHOUGH accurate records have been kept on lobster molting patterns for years, they refer mostly to the age and growth associated with ecdysis (Hadley 1906; Herrick 1911; Wilder 1953; Hughes and Matthiessen 1962). Seasonal synchrony of molting is a familiar phenomenon to fishermen as well as researchers. In Rhode Island peak molt frequences occur in May–June and October–November. The diel pattern of ecdysis, however, is poorly described in the literature. It has long been known that lobsters are nocturnally active (Herrick 1911) and both Herrick and Brook (1887) suggested that *Homarus americanus* exuviates at night. The only two precise records, however, are by Anderton (1909) who noted the ecdysis of an adult male at 1630 and Herrick (1911) who describes a male "shedder" starting ecdysis at 1448. Clearly more data are required for an assessment of the diel patterns of ecdysis in *H. americanus*.

Materials and methods — Egg-bearing female lobsters were obtained from John Hughes from the Massachusetts Lobster Hatchery at Oak Bluffs. Hatched larvae were maintained in communal rearing tanks (Hughes et al. 1974) in a flow-through filtered seawater system. The sea water was drawn from Narragansett Bay and passed through a series of filters: a rapid sand filter, a biological filter composed of gravel and dolomite chips, a fiber cartridge filter, and finally a charcoal filter. The larvae were offered a ration of frozen adult brine shrimp (*Artemia salina*) 3 times daily.

Following ecdysis into the fourth stage, lobsters were removed from the communal rearing tubs and placed into individual compartments (5.2 cm × 5.2 cm × 5.2 cm) of polypropylene containers. In this static system filtered sea water was changed once a day, prior to a single daily food ration of live *A. salina* nauplii. Water changes and feeding times were varied over a 24-h period with approximately one third of the animals fed each 8-h shift. Air, and therefore water temperature, was controlled with an air conditioner ($\bar{x} = 21.5$, SD = 0.5, R = 19.8–22.6 C). Salinity varied from 28.5–30.0‰. Oxygen, NH_4, NO_2, NO_3, and pH were measured daily. Daylight entered the room through a single east-facing window, and an artificially controlled 14L:10D (on at 0600) photoperiod was superimposed on the natural illumination, allowing a natural dawn and dusk along with uniform daytime light intensities. Ecdysis of individual lobsters was noted at 3-h intervals throughout the day, from August 6 through September 4. During the dark phase four 25-W red lights were on continuously, allowing us to take readings, feed the lobsters, and check for fresh casts. Occasional light intrusions resulted from workers entering or leaving the room.

As individuals molted into the fifth stage they were

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assigned to one of five different treatments of an experiment assessing the relative importance of specific sensory modalities on the rate of molting. One of the treatments paired lobsters in a single cell, allowing physical interaction and utilization of all sensory modalities. In the other four treatments animals were paired, but inhibited from physical interaction by a partition constructed of either white or clear plexiglass with holes present or absent, thus allowing possibility of communication by chemical, visual, neither or both pathways. Each experimental animal received a shelter (gray, opaque, hemicylindrical piece of PVC plastic). Frozen adult brine shrimp were offered daily to each lobster, using the same schedule as previously described. All other aspects of the holding conditions were similar to those described for the fourth stage animals, as well as the protocol for changing the water, performing the chemical analyses, and recording the times of ecdysis.

Results — Ecdysis into both fifth and sixth stages occurs primarily during daylight hours with 84.0% of fifth molts and 81.4% of sixth molts coming between 0600–1800 (Fig. 1). The observed frequency distribution in both stages does not fit a normal curve. Inspection of the graph indicates a mode at the 1030 class mark in both stages. The patterns of ecdysis for the two stages are not significantly different ($\alpha = 0.05$, χ^2).

Since the holding conditions differed for the animals used in compiling the sixth stage data, Kendall's coefficient of concordance (W) was calculated to test the similarity in the temporal distribution of ecdysis among animals molting into this stage. Significance of this test may be approximated by a χ^2 distribution (Siegel 1956). The sets of data from the five treatments showed sufficient similarity ($\alpha = 0.05$) to be pooled.

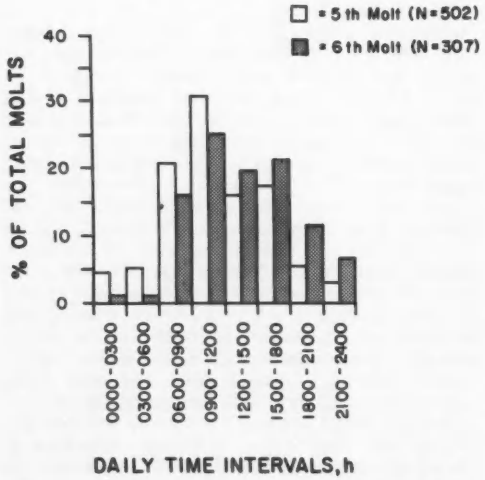

FIG. 1. Diel frequencies of fifth and sixth molts of juvenile *Homarus americanus*. The fifth molt (ecdysis) is considered to occur prior to the fifth stage.

We examined the time of ecdysis for each stage by individuals to see if some tended to molt consistently early in the day and other consistently late. There was no significant tendency for individuals to molt into the fifth and sixth stages at the same time of day ($r = 0.04$).

Discussion — Notwithstanding the old precept that lobsters molt at night, our data show ecdysis to be a diurnal phenomenon. Both of the accurate early records of ecdysis concur with this finding (Anderton 1909; Herrick 1911). The timing of ecdysis, however, differs from that of hatching. Ennis (1973) described female *H. gammarus* releasing larvae on successive nights, shortly after the onset of darkness. This is the only point in the diel period in which hatching occurs. The timing of larval release is a more variable process for *H. americanus* (personal observation). It too is most frequent shortly after dark, but larvae may be released at other times as well.

The times of ecdysis have been reported for several other crustaceans. Peterson (1969), working with the rock lobster, *Jasus lalandii*, witnessed the ecdysis of 11 juvenile and 7 adults (43–100 mm carapace length). The onset ranged from 22 min prior to sunset to 6 h after sunset. Winstanley (1970) observed the ecdysis of a mature female *J. lalandii* starting at 0106. The exuviation of three adult male *J. tristani* occurred approximately 2 h after sunset (Peterson 1969). A single precise record for a captive *Panulirus longipes cygnus* stated 0108 as the onset of ecdysis (Thomas 1966); in addition, Thomas' records showed that of approximately 100 exuviations, all but one were nocturnal. Herrick (1911) has indicated 1500 as the onset of ecdysis in the shrimp, *Alpheus dentipes*. It is intriguing that most of the recorded observations of ecdysis are during a nocturnal phase, contrary to that of *H. americanus*.

In our laboratory studies the changeover from the dark to the light phase may have triggered ecdysis. Other factors in the physical environment such as temperature, salinity, NH_3, NO_2, No_3, and pH were either maintained at a constant level or exhibited no systematic variation which the lobsters might use as a signal to begin ecdysis. Feeding times also varied with respect to the time of day or night and would be a poor consistent signal.

Photoperiodic control of the molt cycle has been demonstrated in crayfish, *Orconectes virilis* (Aiken 1969). Aiken suggested that day length

influences the release of molt-inhibiting hormone (MIH) which in turn controls the production of molting hormones (MH) in the Y-organs of crustaceans (Passano 1960), but Aiken (1969) also reported that *H. americanus* is insensitive to photoperiodic control of its molt cycle. Bliss and Boyer (1964) studied molting in the land crab, *Gecarcinus lateralis*, and found that temperature, photoperiod, moisture, and solitude all could affect the length of the molt cycle.

Light intensity, per se, is not the only factor involved with ecdysis. During daylight hours lobster will molt inside a darkened shelter, if given the opportunity (personal observation), but the timing apparently is not affected by shelter availability. This can be seen by comparing the data from the two stages. In the fourth stage, prior to ecdysis, shelters were not provided whereas they were present during the following stage. Molting patterns were not altered.

Why do lobsters molt during the daytime? Clearly, it must be an adaptive trait. The period of ecdysis and immediately thereafter is the most vulnerable time in a lobster's life. its softened cuticle reduces the animal's ability to defend itself or a shelter. Many of the lobster's predators such as codfish, sharks, skates, and other lobsters (Herrick 1911; Wilder 1957; Stewart 1972) are nocturnal, yet some are not. *Lophius americanus*, the goosefish, (Stewart 1972) and cunners, *Tautogolabrus adspersus*, are diurnally active. Therefore, avoidance of predators is only a partial answer. Another aspect of the lobster's biology must also be considered. The extreme dependence on shelter during the day has been shown in field and laboratory studies (Cobb 1971). In the field lobsters return to their burrows as the light intensity increases. Competition for available shelters among lobsters has been demonstrated (O'Neill 1974) and probably also occurs between *H. Americanus* and *C. borealis* (Fogarty 1975).

For juvenile field populations ecdysis probably occurs about midday, inside shelters. Ecdysis during the day reduces predation and minimizes shelter competition with other crustaceans, allowing a short period of time to recover from the act of ecdysis. Noting this trait, several suggestions may be offered to lobster culturists. Mortalities in mass culture systems might be reduced if adequate shelter is provided. Cobb (1971) suggested suitable shelter dimensions. Secondly, perturbations to the system such as feeding should be withheld until evening. This would minimize locomotor activity, and shelter exploration during the most critical time period for newly molted lobsters.

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An Instrument with Internal Calibration for Monitoring Chlorine Residuals in Natural Waters[1]

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MARINENKO, G., R. J. HUGGETT, AND D. G. FRIEND. 1976. An instrument with internal calibration for monitoring chlorine residuals in natural waters. J. Fish. Res. Board Can. 33: 822–826.

To increase the sensitivity of field instruments for measurement of low level chlorine concentrations in natural waters a new monitor was developed. Iodine, resulting from oxidation of potassium iodide by chlorine residual, is measured amperometrically in a system in which coulometrically generated iodine is used as a system calibrant. Laboratory tests, field tests, and comparisons of the new chlorine monitor with other instruments were performed. Data obtained with the new instrument show that chlorine concentrations down to a few parts per billion can be measured. The instrument is portable, and has built-in electrical calibration features and direct read-out display in either microamperes or parts per million of residual chlorine.

MARINENKO, G., R. J. HUGGETT, AND D. G. FRIEND. 1976. An instrument with internal calibration for monitoring chlorine residuals in natural waters. J. Fish. Res. Board Can. 33: 822–826.

Nous avons mis au point un nouvel appareil de contrôle permettant d'augmenter la sensibilité des instruments utilisés sur le terrain pour la mesure de faibles concentrations de chlore dans des eaux naturelles. L'iode, résultant de l'oxydation de l'iodure de potassium par le chlore residuaire, est mesuré ampérométriquement dans un système dans lequel l'iode engendré coulométriquement est utilisé comme étalon du système. Nous avons fait des essais en laboratoire et sur le terrain, et avons comparé le nouvel appareil de contrôle du chlore avec d'autres instruments. Les données obtenues avec ce nouvel appareil indiquent qu'il est possible de mesurer des concentrations de chlore aussi faibles que quelques p.p. milliard. L'instrument est portatif, possède un dispositif d'étalonnage électrique et un tableau à lecture directe en microampères ou ppm de chlore résiduaire.

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GROWING concern has recently been focused on the environmental effects of chlorine and chlorine derivatives in aqueous ecosystems (Brungs 1973; Arthur and Eaton 1971; Tsai 1968, 1973; Virginia State Water Control Board 1974). Sewage treatment plants (STP's) and power plants are principal contributors of these contaminants to natural water systems. In STP's, chlorine is used to disinfect the sewage and decrease biochemical oxygen demand, while in the power plants it is generally used to control fouling in heat exchangers. The contribution of chlorine from power plants may be significant but it is overshadowed by the large quantities entering the environment from STP's. For instance, in the State of Virginia, regulations require at least 2 ppm chlorine residual to be maintained in sewage effluents entering the receiving streams. This practice has caused massive fish kills in the James River estuary in 1973–74 (Virginia State Water Control Board 1974).

Investigations of chlorine-induced fish kills have been hampered by the fact that the existing analytical methods for field use are not sensitive enough to measure chlorine residuals in the river

[1]Constructional details of the chlorine monitor, including working drawings, are available from G. M.

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below levels of 0.1 ppm. This paper reports a new method of measurement which has a sensitivity of parts per billion.

It has been recognized that the measurement of total residual chlorine in natural waters at a level of few 10ths of a ppm can best be performed using commercial instruments that employ amperometric titration (Brungs 1973). Some of these instruments are based on the titration of iodine, liberated upon addition of a buffered KI solution to a measured volume of the water sample, with a standard solution of a reducing agent, such as As (III). The end point of titration is determined amperometrically. In field use these devices are capable of furnishing rapid estimates of chlorine concentrations from the total volume of titrant required to reach an end point. Such field titrations are usually performed under less than ideal conditions, e.g., on shipboard. The sensitivity of the presently available, commercial chlorine detectors falls short of the desired low-level chlorine measurements thought to be responsible for damage to fish. Furthermore, preparation of standard solutions in the field is not convenient, since prolonged storage of standard As (III) solutions is subject to errors due to slow oxidation of trivalent arsenic by air oxygen. Also, in studies of natural waters, collection and subsequent shipment of samples back to the laboratory for analysis is not suitable when dealing with low concentrations of volatile and reactive components such as chlorine.

The limitations of the existing methods for low level measurement of chlorine in natural waters prompted the work on the development of a new chlorine monitor. The ultimate aim was to develop an instrument capable of continuous monitoring of chlorine concentrations in natural waters at subpart per million concentration levels.

Chlorine monitor design — Solid electrode amperometry (e.g. using platinum microelectrodes) is used predominantly as an end point detection technique (Lingane 1958). In amperometry, an electrical potential is impressed upon a cell consisting of a polarizable microelectrode and a nonpolarizable reference electrode in order to make the cell respond to a particular constituent in solution. A current is produced that is proportional to the concentration of the selected constituent. In the case of chlorine using a Pt microelectrode and a calomel reference electrode, this potential happens to be 0 V, i.e. the cell acts as a battery. Even though the current produced is proportional to the concentration of chlorine, the system must be calibrated with standard solutions to determine the chlorine concentration.

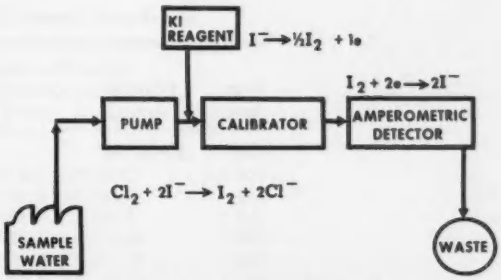

FIG. 1. Schematic diagram of NBS chlorine monitor.

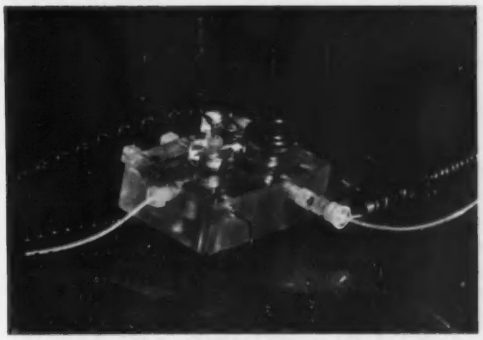

FIG. 2. NBS chlorine monitor.

With this in mind an internal calibration system was designed, consisting of a coulometric cell which generates the desired electroactive species in situ at known concentrations.

To combine the electrochemical features with a continuous constant flow sampler into a monitoring system, the scheme outlined in Fig. 1 and described below was devised. The system is based on the addition of iodide to the sample, buffered at pH 4. If chlorine–chloramines are present in the sample, a stoichiometric amount of iodine is produced by the oxidation of iodide to iodine.

The amperometric detector for monitoring the iodine concentration consists of a Pt microelectrode (about 3 cm^2 in area) and a saturated calomel electrode. A current is produced in the detector cell that is proportional to the concentration of iodine, and thus to that of chlorine.

For calibration purposes, an accurately known amount of iodine is generated by coulometrically oxidizing iodide to iodine in the calibrator cell, the result being exactly the same as the reaction of chlorine with iodide in aqueous media.

The electrochemical chlorine flux-monitoring system is shown in Fig. 2. It consists of two

TABLE 1. Serial dilution of tap water.

Expt no.	Dilution factor	Calculated concn Cl_2 (*ppm*)[a]	Measured concn Cl_2 (*ppm*)	dev
1	Undiluted	—	1.237	
2	2	0.618	0.620	+0.002
3	4	0.309	0.298	−0.011
4	8	0.154	0.148	+0.006
5	16	0.077	0.072	−0.005
6	32	0.038	0.038	0.000
7	64	0.019	0.020	+0.001
8	128	0.010	0.010	0.000
9	256	0.005	0.005	0.000
10	512	0.002	0.003	+0.001
11	1024	0.001	0.001	0.000
			$\overline{\text{dev}}$ =	$\overline{\pm 0.003}$

[a]Based on the measured Cl_2 concentration in undiluted tap water in Experiment no. 1.

distinct and separate electrochemical cells, interconnected in a single unit, which function independently: the calibration cell and the detection cell. In the system, 0.1 M KI solution in a pH 4 buffer and saturated with NaCl is slowly injected into the stream at a constant rate. The purpose of adding NaCl is to increase the electrical conductivity of the water sample, since electrical current must flow in the calibrator as well as in the detector.

The calibration cell operates only during calibration of the instrument, which can be performed as frequently as desired. Insignificant changes of the calibration have been found to occur in the course of a working day. The function of this cell is to generate a known, preselected flux of iodine for calibration of the detector cell signal.

The chlorine monitor system requires the water sample to flow at a constant, known rate (e.g. 1 ml/s) through the monitor so that the ratio of electrically generated iodine flux to the water flux corresponds to the concentration of iodine. This water flux must be known and remain constant. When the water sample is pumped through the system at a constant rate, the output of the detector cell is electrical current, the magnitude of which depends on the concentration of chlorine. Typically, for our system the current is 20 μA when the concentration of chlorine in the sample is 1 ppm.

An electronic measurement package was designed and constructed to complement the electrochemical chlorine monitor cell (R. Shideler, National Bureau of Standards, Washington, D.C. personal communication). The instrument fulfills the requirements for a field instrument. It is either battery or line operated, portable, and features a digital display of the signal in ppm of chlorine after calibration.

Performance of chlorine monitor — Laboratory — Two series of experiments were conducted with the chlorine monitor to assess its sensitivity in the measurement of low levels of chlorine and to estimate its accuracy.

To study the sensitivity of the chlorine monitor in a laboratory accessible system, cold tap water was selected as the system to be investigated. The chlorine concentration was measured in undiluted tap water (after clearing the lines of stagnant water by running it for 1 h before sampling). Subsequently, the tap water was quantitatively diluted with distilled water and the concentration of chlorine was measured after each dilution. The instrument's response was linear over three orders of magnitude of concentration change with a sensitivity of a few ppb of chlorine (Table 1).

To estimate the accuracy of the measurement, a fresh bottle of commercial bleach was used to prepare a 52-ppm stock solution. Subsequently, this solution was diluted just before measurement, according to the scheme used in the tap water experiment. All dilutions were made with 1% accuracy. The results of this series of measurements are shown in Table 2. Measurements of chlorine concentration in the 100–10 ppb range indicate that an overall accuracy of 1 ppb may be achieved. The accuracy of the method and the instrument will be assessed further by more exhaustive tests.

Field — Two separate field tests of the chlorine monitor were performed. The first test was per-

TABLE 2. Serial dilution of a commercial bleach.

Dilution of 52 ppm bleach solution	Calculated concn Cl_2 (*ppm*)	Measured concn Cl_2 (*ppm*)	dev
1:1000	0.052	0.051	−0.001
1:2000	0.026	0.025	−0.001
1:4000	0.013	0.011	−0.002
1:800	0.006	0.005	−0.001
1:500	0.104	0.104	0.000
1:1000	0.052	0.053	+0.001
1:2000	0.026	0.026	0.000
1:4000	0.013	0.013	0.000
			$\overline{\text{dev}} = 0.001$

TABLE 3. Field test near STP during a chlorine residual-caused fish kill.

June 30, 1974		NBS–VIMS chlorine via flux-monitor (*ppm*)[a]	WCB chlorine (*ppm*)[a]
Time	Location		
11:00	STP outfall	0.160	—
11:20	Midriver channel at Blunt Point	0.022	—
11:35	Buoy N12	0.029	—
12:00	STP outfall	0.223	0.20
12:20	STP outfall	0.245	0.24
12:30	STP outfall	0.252	0.23
12:55	200 m east of outfall	0.035	—ND[b]
13:20	1000 m east of outfall	0.029	—ND
13:35	STP outfall	0.181	0.21

[a]Concentrations reported as combined residuals.
[b]ND = not determinable by technique employed.

formed in an actual emergency situation at the peak of a fish kill in the James River near Newport News, Virginia, on May 30, 1974. The measurements were conducted aboard a Virginia Institute of Marine Sciences research vessel covering a several-kilometer area of the river near the outfall of a sewage treatment plant. Initially the boat was the only one from which measurements were performed. Subsequently, we were joined by Virginia State Water Control Board (WCB) personnel, equipped with a commercial amperometric titrator. The results of this river survey are given in Table 3. For those measurements made by both instruments, agreement was good.

At the request of the State of Maryland Water Resources Administration, a second field test was made on June 19, 1974 in streams of northern Maryland. A conventional amperometric instrument was also used in addition to the chlorine flux monitor to determine the chlorine in the same water.

At one point an undiluted effluent from a small sewage treatment plant which services a housing development in northern Maryland was sampled. In this case the amount of chlorine was too great to provide a confident value using the commercial instrument. Several burette fillings of the As (III) solution were required, and the estimate of chlorine concentration obtained was about 4.5 ppm. The chlorine flux monitor displayed 5.57 ppm as the measured concentration of chlorine. A sample of stream water taken a few meters away from the outfall was also analyzed using both instruments. The chlorine monitor value was 0.028 ppm and that obtained using the titrator was 0.03 ppm.

Acknowledgments — We gratefully acknowledge the contributions of Dr J. R. McNesby of the Office of Air and Water Measurement at the National Bureau of Standards.

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Homing of Artificially Imprinted Steelhead (Rainbow) Trout, *Salmo gairdneri*

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COOPER, J. C., AND A. T. SCHOLZ. 1976. Homing of artificially imprinted steelhead (rainbow) trout, *Salmo gairdneri*. J. Fish. Res. Board Can. 33: 826–829.

Greater numbers of rainbow trout (*Salmo gairdneri*) exposed to morpholine as fingerlings as compared to trout left unexposed to morpholine as fingerlings homed to a stream scented with this odor (174 vs. 16). In order to locate this stream, the imprinted fish are able to search a distance of at least 13 km. These data support the olfactory hypothesis of homing.

COOPER, J. C., AND A. T. SCHOLZ. 1976. Homing of artificially imprinted steelhead (rainbow) trout, *Salmo gairdneri*. J. Fish. Res. Board Can. 33: 826–829.

Les truites arc-en-ciel (*Salmo gairdneri*) exposées à la morpholine au stade d'alevins de la grosseur du doigt se dirigent en plus grand nombre vers un cours d'eau imprégné de cette odeur (174 *vs* 16), comparativement à des truites de la même taille qui n'ont pas été exposées à la morpholine. Les poissons ainsi marqués peuvent localiser ce cours d'eau à une distance d'au moins 13 km. Ces résultats confirment l'hypothèse olfactive du retour à la rivière natale.

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IT has been hypothesized that salmonids store odor information about the stream during the early stages of their life (i.e. imprint to the homestream odors) and recall this information during the stream phase of the reproductive migration (Hasler 1966). Earlier reports (Cooper and Hasler 1973, 1974, 1975; Cooper 1974; Cooper et al. 1976; Madison et al. 1973; Scholz et al. 1973) concluded that coho salmon (*Oncorhynchus kisutch*) can imprint to a synthetic odor, morpholine, retain this information through the spawning migration, and use it to identify a stream scented with this chemical, as proposed earlier by Hasler and Wisby (1951). It has been extensively documented that an anadromous variety of the steelhead, rainbow trout (*Salmo gairdneri*) homes to the stream of origin or stream of release (Lindsey et al. 1959; Northcote 1962; Taft and Shapovalov 1938; Wagner 1969) in a manner similar to that of salmon. The purpose of the research reported here was to determine if rainbow trout could be imprinted to a synthetic odor.

In 1972, three groups of 3000 hatchery-raised rainbow trout fingerlings each were held in three separate tanks at Oak Creek, South Milwaukee, Wisconsin, from April 15 to May 12. One group was exposed to morpholine at a concentration of 5×10^{-5} mg/liter, while the other two were left unexposed as controls. The water in the tanks was pumped from 0.75 km out in Lake Michigan and was considered neutral because it did not contain odors associated with any stream. All groups were marked by finclipping. The exposed group and a control group were released at Milwaukee Harbor Breakwater, 13 km north of Oak Creek. A second control group was released at Oak Creek (Fig. 1, Cooper et al. 1976). During fall 1972, fall and spring 1973, and spring 1974, morpholine was released into Oak Creek to give a concentration of about 5×10^{-5} mg/liter in the stream, the concentration used for imprinting. These experiments were repeated in 1973 with two groups of 3900 fish each. One group was exposed to morpholine for 1 mo at Oak Creek

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TABLE 1. Census of morpholine-imprinted and nonimprinted (control) fish at Oak Creek, 1972–74. Morpholine was used for imprinting the experimental group. Finclips are: RP = right pectoral, LP = left pectoral, LM = left maxillary, D = dorsal. MB = Milwaukee Breakwater, OC = Oak Creek morpholine.

Exptl group	Finclip	No. Released	Release site	No. Captured fall 1972	No. captured spring 1973	No. captured fall 1973	No. captured spring 1974	Total no. captured	% captured of no. released
					1972				
Imprinted	RP	3000	MB	12	19	67	76	174	5.8
Control	LM	3000	MB	0	1	4	11	16	.53
Control	LP	3000	OC	21	15	14	8	58	1.9
Strays				80	70	123	—	—	—
					1973				
Imprinted	D-LP	2000	MB	—	—	18	48	66	3.3
Control	D-RP	3900	MB	—	—	1	7	8	.21

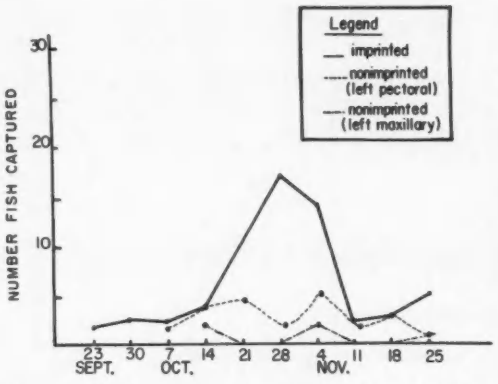

FIG. 1. Catch curve for rainbow trout (*Salmo gairdneri*).

Water Filtration Plant, while a second group was left unexposed. Because 1900 of the exposed groups were accidentally killed in transit, only 2000 fish were released at the Milwaukee Harbor Breakwater.

With coho salmon, the smolting period was considered appropriate for artificial imprinting (Cooper et al. 1976; Cooper and Hasler 1973, 1974; Madison et al. 1973; Scholz et al. 1973, 1975). In these experiments, salmon were exposed for approximately 2 wk before until 2 wk after the first signs of smolting behavior. Because rainbow trout showed few signs of smolting during the period of morpholine exposure, it was not possible to determine exactly the sensitive period by this means. The fish did not show color changes but did exhibit some downstream orientation (negative rheotaxis). This in contrast to earlier studies (Robertson 1948, 1949) that indicate color changes occur in this species. Exposure was in early spring since it is the normal time for downstream migration of young steelhead trout. To determine the number of returning spawners, the stream was censused in spring and fall by several methods; electrofishing, creel census, and gillnetting. See Cooper et al. (1976) for further details.

Apparently, rainbow trout can be imprinted to morpholine and use this information to home to a stream scented with this odor. This species may home successfully by olfaction after a search over a distance of at least 13 km. For the fish released in 1972, 174 experimental compared to 16 control fish were captured, a ratio of about 11:1 (Table 1). This difference is significant ($\chi^2 = 80$, $P \leqslant 0.01$). Sixty-six experimentals and eight controls returned to the stream from the 1973 release. The number of fish returning in this experiment is smaller than for the 1972 release, since a second year of census data were not available. The exposed group and one unexposed group of trout from the 1972 release returned to Oak Creek in a roughly normal distribution with respect to time with a peak on October 28 (Fig. 1). Too few of the second control group returned to determine the shape of the distribution.

Fifty-eight trout from the second control group (released near Oak Creek in 1972) were captured. Little information can be obtained from this group, since a corresponding imprinted group was not released along with them near Oak Creek. Hasler (1966) and Harden-Jones (1968) have divided the homing migration into migration in and near the homestream. At the end of the first phase, the fish search for odors of the homestream. Since this group returned to Oak Creek, a water to which they had no previous exposure during smolting, it may be that they were able to return to the general area of Oak Creek by some mechanism (other than olfaction) associated with the search of phase of homing. Because Oak Creek was the only stream in the immediate area, the trout chose to enter this river. Note that smaller numbers of this control group were captured (58) compared to the morpholine-imprinted group (174). There is a possibility that these fish imprinted to the Oak Creek area water at the time of their release, although earlier work with coho indicates that exposure to stream odors after smolting is far less effective than exposure to artificial odors during the smolting period (Madison et al. 1973; Cooper 1974; Scholz et al. 1973, 1975).

We conclude that rainbow trout can be imprinted to morpholine and can use this olfactory information to find a stream with which they have no previous experience, provided the stream has been scented with this odor. In order to locate the stream, imprinted fish are able to search a distance of at least 13 km.

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Distribution and Timing of Tag Recoveries from Native and Nonnative Atlantic Salmon (*Salmo salar*) Released into Big Salmon River, New Brunswick

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JESSOP, B. M. 1976. Distribution and timing of tag recoveries from native and nonnative Atlantic salmon (*Salmo salar*) released into Big Salmon River, New Brunswick. J. Fish. Res. Board Can. 33: 829–833.

Recaptures of tagged Atlantic salmon (*Salmo salar*) released as smolts into the Big Salmon River varied between annual releases with no differences found on the basis of native or nonnative origin. Few were recaptured outside the Bay of Fundy. Distant fisheries in Greenland and Newfoundland take few salmon from Big Salmon River and commercial exploitation within the Bay of Fundy is minor, particularly by the former large fishery off the mouth of the Saint John River.

JESSOP, B. M. 1976. Distribution and timing of tag recoveries from native and nonnative Atlantic salmon (*Salmo salar*) released into Big Salmon River, New Brunswick. J. Fish. Res. Board Can. 33: 829–833.

Les mises en liberté annuelles dans la Grande rivière au Saumon de smolts de saumon atlantique (*Salmo salar*) marqués donnent des taux de recapture variables, sans différences entre les smolts indigènes et non indigènes. Il y en a peu de recapturés en dehors de la baie de Fundy. Les pêcheries lointaines du Grœnland et de Terre-Neuve capturent peu de saumons provenant de la Grande rivière au Saumon, et l'exploitation commerciale à l'intérieur de la baie de Fundy est mineure, en particulier celle de la pêcherie, jadis importante, de l'embouchure de la rivière Saint-Jean.

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THE marine migration of Atlantic salmon (*Salmo salar*) originating in Bay of Fundy rivers is poorly known. Huntsman (1931), writing primarily of Saint John River salmon, hypothetized that Bay of Fundy salmon remain within the Bay during their marine phase. The operation from 1963 to 1971 of a counting fence on the Big Salmon River permitted the collection of data on the location of recapture of tagged salmon of native and of nonnative origin released there.

The Big Salmon River drains an area of approximately 332 km² and discharges into the New Brunswick side of the Bay of Fundy (Fig. 1). Counts of ascending adult salmon at the fence have averaged 2200 fish between 1964 and 1972 (range 700–4600).

Tagging methods — Tagged wild native salmon smolts and/or hatchery-reared nonnative smolts were released in various numbers into the Big Salmon River from 1963 to 1971; 115,896 smolts in total (Table 1). Nonnative smolts were reared at a fish culture station near Saint John, New Brunswick, and were of northern New Brunswick stock, either the Northwest Miramichi River (1963–66 releases) or the Restigouche River (1968–69 releases). Releases of nonnative smolts were made close inside the river mouth in 1963 and 1964 and farther upstream in 1965 and 1966. In 1968 and 1969, approximately half of the total release was made about 22.5 km upstream with the remainder released inside the river mouth. Native smolts were tagged and released daily during periods of smolt descent at the counting fence near the river mouth.

Thirty to 45% of the counted native run was tagged yearly. Modified Carlin tags were attached to smolts with black monofilament polyethylene (Saunders 1968), except in 1963 when multicolored beads on a monofilament tie were used.

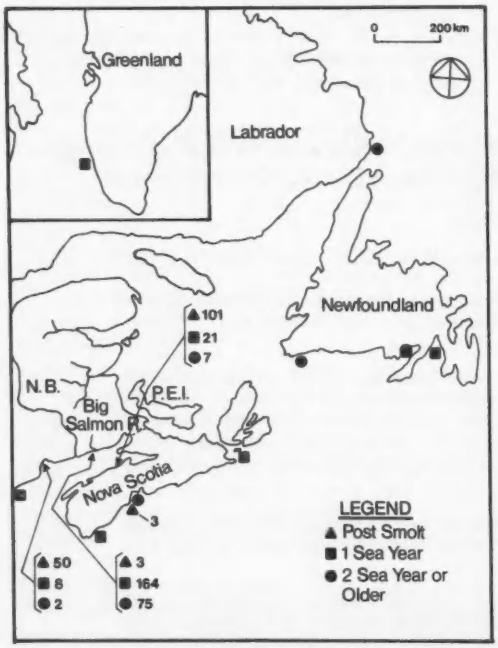

FIG. 1. Distribution and number of tag returns, by age-group, of Atlantic salmon (*Salmo salar*) as smolts in the Big Salmon River, N.B., from 1963 to 1971. The number beside a symbol indicates the number of recaptures at that approximate location. Where no number is used, only one recapture is indicated.

Recapture of tagged fish — Tagged post-smolt and adult salmon were recovered in various commercial fisheries, at the counting fence and by angling within the river. A reward was given for the return of tags and information on fish length, weight, sex and date, location, and method of capture.

Total recovery of tagged native and nonnative Atlantic salmon released in a given year ranged from 0.1 to 2.0% and from 0.1 to 0.6%, respectively (Table 1). Post-smolts (young salmon that have not yet spent one winter at sea) of both stocks were recaptured in similar percentages. Older salmon recaptures were relatively more numerous for native than nonnative stocks (0.47 vs. 0.17%). Comparison of the percent recovery of fish tagged in the years 1968–69, from both native and nonnative origin smolt releases, shows that while more nonnative than native post-smolts were recovered (0.25 vs. 0.07%), older salmon recaptures were again relatively more numerous for native than nonnative stocks (0.12 vs. 0.00%).

Approximately 99.2% of the native recaptures as compared with 96.6% of the nonnative recaptures were from within the Bay of Fundy (Big Salmon River included) (Fig. 1). More than twice as many Atlantic salmon in each of the post-smolt, 1, and 2+ sea-year age-groups were recovered on the Nova Scotian shore within the Bay of Fundy as on the New Brunswick shore (excluding home river returns). The year-to-year variability in numbers of fish recaptured at each location makes it impossible to ascertain the significance of the difference. No native post-smolts were taken in the St. Margaret's Bay–Mahone Bay area of Nova Scotia's Atlantic coast. About 93% of 1 and 2 sea-year native salmon returns were from the home river as compared to 83% of nonnative returns. Two native fish were taken

TABLE 1. Recaptures of tagged Atlantic salmon (*Salmo salar*) released as smolts into the Big Salmon River from 1963 to 1971.

Release year	No. of tagged smolts released		Recaptures of post-smolts		Recaptures of 1 sea-year fish		Recaptures of 2+ sea-year fish		Total recovery			
									No.	%	No.	%
	Native	Nonnative[a]	Native	Nonnative	Native	Nonnative	Native	Nonnative	Native		Nonnative	
1963	—	10,000	—	0	—	4	—	6	—	—	10	0.1
1964	—	17,766	—	15	—	14	—	6	—	—	35	0.2
1965	—	19,937	—	30	—	71	—	23	—	—	124	0.6
1966	—	17,979	—	27	—	17	—	4	—	—	48	0.3
1967	3,900	—	1	—	37	—	40	—	78	2.0	—	—
1968	5,120	9,969	3	23	3	0	3	0	9	0.2	23	0.2
1969	3,792	9,783	3	27	4	0	1	0	8	0.2	27	0.3
1970	9,864	—	6	—	5	—	1	—	12	0.1	—	—
1971	7,786	—	22	—	44	—	5	—	71	0.9	—	—
Total	30,462	85,434	35	122	93	106	50	39	178	0.58	267	0.31
% Total			0.11	0.14	0.31	0.12	0.16	0.05				
							Mean		35.6	0.68	44.5	0.28
							SD		35.63		40.94	

[a]Nonnative smolt releases were of Northwest Miramichi River stock (1963–66) and Restigouche River stock (1968–69).

outside the Bay, one at Port-aux-Basques, Nfld., and the other near Boston, Mass. The seven nonnative recaptures from outside the Bay were recovered at locations from eastern Nova Scotia to West Greenland.

Timing of recaptures — Within the Bay of Fundy, native and nonnative post-smolts were recaptured mostly during July and August in both the Passamaquoddy area (near the international border) and along the Nova Scotia coast (Fig. 1). Release location of nonnative smolts within the river had no significant effect upon the mean time to recovery. Within years, there was no apparent difference in recovery dates of native and nonnative post-smolts either within or between recovery areas. For example, of the 14 native post-smolt recoveries in 1971 for which recapture dates are available, four were taken on the Nova Scotia coast an average of 57 days from release (range 46–69), while eight were captured in the Passamaquoddy area an average of 61 days from release (range 47–77 days). The mean times to recovery are not significantly different ($P > 0.05$). One post-smolt capture in the Passamaquoddy region was caught 95, and another 98 days after release. Between years, recapture dates and dispersal of both native and nonnative post-smolts varied considerably but not always significantly. In 1964 and 1968, individual nonnative post-smolts were recaptured outside the Bay more than 480 km from the Big Salmon River and approximately 82, 59, and 52 days respectively, after release.

Most Atlantic salmon are caught in the Bay of Fundy from June through September with returns to the Big Salmon River occurring from late June to early November, peaking between late August and early September.

Exceptions to the pattern of salmon recaptures include two post-smolts seined in the Passamaquoddy area herring fishery in January, seven adult salmon taken outside the Bay during June and July off Newfoundland and along the Atlantic coast of Nova Scotia, and two 1 sea-year salmon taken in September, one off Newfoundland and the other off West Greenland.

Discussion and conclusions — Recaptures of tagged Atlantic salmon released as smolts varied from year to year but no difference was ascribable on the basis of stock origin when all recaptures were considered. Comparison of the 1968–69 returns when both native and nonnative fish were released supports this conclusion. Native fish showed greater survival to grilse and older stages. Ritter (1975) suggests that increased survival of native relative to nonnative stock reflects a clinal decrease in survival with increasing distance between donor and recipient streams. In this case, the distance exceeds 800 km of coastline.

Neither native nor nonnative tagged salmon were recaptured in appreciable numbers outside the Bay of Fundy. In contrast, the early Pollet River studies in using hatchery-reared smolts of Miramichi River stock produced many widely scattered recaptures outside the Bay of Fundy (Kerswill 1954). The use of nonnative stock may not be valid for assessing the movement and distribution of native stock (Ritter 1972), but this study notes little difference in the recapture dates or locations between fish of different origin released from Big Salmon River.

Since the Greenland fishery exploits mainly salmon remaining at least two winters at sea before maturing (Allen and Saunders 1967) and approximately 93% (4-yr mean) of all virgin fish from the Big Salmon River spawn as grilse, and grilse have composed from 14.6 to 68.5% of the total return, it is unlikely that this stock contributes in any important way to this fishery. The single tag recovery off West Greenland was from a 1 sea-winter fish of nonnative origin. Of the four 1 and 2 sea-year salmon recovered off Newfoundland, only one was of native stock (0.7% of total native salmon recoveries) and it is doubtful that native salmon contribute significantly to this latter fishery. No tagged salmon native to the Big Salmon River have been recovered along the Atlantic coast of Nova Scotia, although salmon of Saint John River origin have been taken (C. Wykes, personal communication). Big Salmon River salmon apparently contribute little, if at all, to that fishery. Neither do they contribute importantly to the extensive salmon driftnet fishery off the mouth of the Saint John River since only two tagged, Big Salmon River fish have been reported from this fishery and only three of the 935 fish tagged in driftnetting studies off the Saint John River mouth in 1971 and 1972 were subsequently recaptured at Big Salmon River. Indirect evidence for the low exploitation of this stock by any commercial fishery is the high incidence of annual spawning of previously spawned fish (27.9–62.7%), some of which have spawned 5 or 6 times (Ducharme 1969). Fishing mortality is largely accounted for by angling, which annually catches approximately 30% (mean 1967–72) of the total river return.

The generally low recapture rate of Atlantic salmon tagged and released as smolts reported by Allen and Saunders (1967), May (1971), and Ritter (1972), as well as in this study and the great variability in the number of recaptures be-

tween years in this study tends to obscure any real differences that may exist between native and nonnative stocks. Differences may arise also from factors such as tag loss, nonreporting of tags (Allen and Saunders 1967), and variability in the size, age, and sex composition of the tagged smolts resulting in variable ocean survival (Carlin 1968).

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System for Sampling Large Trawl Catches of Research Vessels

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HUGHES, S. E. 1976. System for sampling large trawl catches of research vessels. J. Fish. Res. Board Can. 33: 833–839.

Procedures and sampling gear designed 1) to sample large research trawl catches prior to sorting by species and 2) to reduce catch handling time and permit an increase in the number of hauls per cruise, are discussed and illustrated. The rationale behind the method relies on the hypothesis that species and size stratification in the codend of the trawl are bilaterally symmetrical. The codend is positioned above a deck bin prior to releasing the catch so that a sampling net can be lifted from the deck bin to remove about one half of the total catch. The sampled portion is processed on a fish sorting table, and the unused portion brailed overboard by a net which lines the entire deck bin. Tests conducted in Puget Sound indicated that the species composition and length distribution in the sample adequately represented the total catch; errors greater than 25% were usually restricted to species composing less than 5% of the catch, and for dominant species errors were less than 10%.

HUGHES, S. E. 1976. System for sampling large trawl catches of research vessels. J. Fish. Res. Board Can. 33: 833–839.

Nous décrivons et figurons des méthodes et des engins d'échantillonnage permettant de 1) prélever des échantillons à même de grosses prises de chalut de recherche avant le triage par espèce et 2) réduire le temps de manipulation de la prise et augmenter ainsi le nombre de traits de chalut par croisière. La méthode repose sur l'hypothèse que la stratification par espèce et par taille dans la poche du chalut a une symétrie bilatérale. Supposant que la composition en espèces et en tailles d'un côté ou de l'autre de la poche est représentative de la prise totale, la poche est mise en place au-dessus d'un compartiment de pont avant de laisser tomber la prise, de sorte qu'un filet d'échantillonnage peut ensuite être soulevé du compartiment, prélevant ainsi environ

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la moitié de la prise totale. La portion échantillonnée est placée dans une table de triage pour traitement. La portion non utilisée est immédiatement rejetée à la mer à l'aide d'un filet qui garnit tout l'intérieur du compartiment de pont. Des essais effectués dans le Puget Sound indiquent que la composition en espèces de l'échantillon représente adéquatement la prise totale. A une exception près, les écarts dépassant 25% se limitent à des espèces constituant moins de 5% de la prise, et, pour les espèces dominantes, les écarts sont inférieurs à 10%. La répartition des longueurs dans la portion échantillonnée est représentative (niveau de probabilité de 10%) des prises totales.

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ASSESSMENT of demersal fish communities to determine species composition, biological information on species, and potential yield is often based upon catches obtained during trawl surveys by research vessels. Two sampling problems are encountered during such surveys. The first concerns use of gear for obtaining catches that are representative of available populations. The second, and subject of this report, concerns the on-deck sampling of large trawl catches at sea.

Procedures for sampling trawl catches at sea have rarely been described in the literature. However, most authorities agree that in addition to being verified by empirical testing, methods of sampling must be suited to vessel facilities and rapid enough so that continued survey operations are not impeded. Large catches have traditionally placed additional burdens on sampling procedures. Paloheimo and Dickie (1963) and Pope (1963) described methods that proved impractical for large catches. May and Hodder (1966) and Westrheim (1967) have reported deck sampling methods that are apparently well suited to their vessels, manpower, and data requirements, but require that the entire catch be sorted by species prior to selection of subsamples for obtaining biological information.

This report describes gear and procedures used to remove approximately one half (a 45–55% portion) of a large trawl catch prior to sorting by species. It is based upon the assumption that size and species stratification observed in deck bins of mixed groundfish occurs in a predictable manner that such stratification can be preserved for the purpose of subsampling.

Methods — Rationale — It is commonly recognized that one problem in obtaining random samples from a pile of trawl-caught fish is the partial segregation or stratification of fish by size. Paloheimo and Dickie (1963) and Westrheim (1967) have noted that larger fish were located near the top of the pile. The former authors suggest that this stratification by size may result from disturbances in the pile causing the smaller fish to slide toward the bottom.

Based upon both underwater and deck observations, I believe that stratification of fish by size and species, commonly observed on deck, is primarily a result of stratification that has occurred in the codend during fishing and retrieval. Observations by myself and other scuba divers at NMFS's Northwest Fisheries Center (High 1969) and a report by Jones (1956) indicate that fish are herded along in front of the footrope during trawling and that individuals drift back into the net after a period of time. Smaller fish, particularly flatfish, which tend to tire more rapidly during the herding process than the larger fish, drop back into the net sooner. Consequently, mean size of a species located in the back portion of the codend will be less than those in the front portion of the codend.

Stratification of species additionally occurs as a result of behavioral variations in response to the fishing net. Once inside the mouth of the trawl, flatfish tend to move rapidly to the back portion of the codend where they attempt to escape by periodically surging forward. Roundfish and rockfish, however, do not become as packed in the codend as flatfish. They commonly enter the after-intermediate or codend section of the net and swim to various degrees with the net during fishing.

Finally, differences in buoyancy also cause stratification of species in the net. During net retrieval, fish with closed air bladders become more positively buoyant and generally move to the upper section of the codend resulting in further segregation. Thus, as the codend is lifted aboard and dumped, behavior and buoyancy result in a pile of fish that is stratified by both size and species.

Thus, it would seem reasonable to expect that the codend fills in a bilaterally symmetrical manner. When viewed in the fishing position, species and size composition found in the left side of the codend should be very similar to the right side. Such symmetry should be maintained in the deck bin if the codend is properly oriented prior to dumping. Thus, even with complex species and size stratification, it should be possible to remove fish from one side of a fish bin mechanically and to use that portion for determining catch com-

position and other biological data which are representative of the total catch.

Sampling gear — Empirical testing of the catch sampling system were conducted in Puget Sound from the 28-m long NOAA fishery research vessel *John N. Cobb* using a 400-mesh Eastern otter trawl with a 31.8-mm liner. The trawl was marked to identify the proper position of the codend prior to releasing the catch into a deck bin.

Deck gear consisted of a 2250-kg capacity dynamometer, a sturdy wooden table for sorting and processing up to 1200 kg of fish, 141-kg capacity weighing scale, 24 bushel baskets that were numbered, and two 63.5-mm mesh nets placed in the deck bin. The first net, called a retainer net, served to line the entire deck bin while the second, the sampling or cargo net, was placed in the deck bin so that it covered about one half of the retainer net.

Procedure — Catch handling can be divided into three steps. First, the codend was lifted aboard, weighed with the dynamometer, properly oriented, and the catch released into the fish bin. Second, the sampling net was lifted from the bin, thereby removing the sample. Sorting by species, and processing the catch was the final step. During the testing period, the remaining portion of the catch was removed from the fish bin with the retainer net and handled as a separate sample, thus providing information on the total catch.

Sorting of species was done from the table. Basically, this was conducted in the manner described by May and Hodder (1966). For dominant species, which would later require subsampling prior to processing, three or four baskets were placed on the table and filled simultaneously by each man rotating from basket to basket. Once filled, baskets were located on deck as a group and the procedure repeated until all individuals of the dominant species were sorted into baskets.

One or more baskets from each group were selected at random by drawing from numbered cards. The number of baskets in each group and number selected from each group were determined to provide at least a 300-fish sample for processing. All baskets of the catch were weighed to the nearest 0.5 kg, and all fish of less abundant species and those in the selected baskets were measured (nearest centimeter-fork length) and tallied on washable plastic strips (Davenport and Harling 1965). Data were later transcribed to permanent forms for ADP processing.

Species and size composition data were determined for the total catch by combining data from cargo and retainer net samples. Cargo net samples were compared to the total catch using chi-square analysis for species composition and cumulative length frequencies (percent) for size composition.

Operation of mechanical sampling system — Traditional methods of trawl retrieval are not affected by the trawl catch sampling system. Sampling and retainer nets are placed in the fish bin where they create no inconvenience. Before lifting the codend aboard, the catch magnitude is estimated; if it appears to be considerably greater than 1200 kg, the cargo net is positioned to sample approximately half of the catch. Once aboard, each split in the codend is weighed and positioned so the top and bottom are athwartship. A line is used to maintain this position and secure the codend against the vessel bulwarks as the net is raised and catch released (Fig. 1A). The cargo net is lifted thereby removing the sample (Fig. 1B) which is then spilled onto the sorting table through a pursed opening. Before sorting the sampled portion, the unneeded portion of the catch is rolled overboard using the retainer net as an apron brailer (Fig. 1C). This practice requires about 20 s to complete and thereby should minimize mortality to crab and most fish without closed air bladders. Operating efficiency is also improved since manpower is not required to clear the fish bin. Sorting from the table is primarily a convenience and further reduces the sorting time. Filled sampling baskets are placed near the stern. After the catch components are weighed and subsampled, if necessary, the sampled fish are processed on the sorting table. Removable rails and the ability to divide the table into bins for research activities further increase operating efficiency. During our surveys, this normally allows two biologists to measure groundfish of secondary importance while two others determine the sex of the target species in preparation for detailed processing.

Empirical test results — Eight tows were completed in Puget Sound during the testing phase. Five catches, ranging from 694 to 1973 kg were mechanically sampled using the cargo net. In each case, the cargo net sample and remainder of the catch were processed separately to determine species and size composition for the sampled portion and (by combining data) for the total catch. The sample net portion ranged from 45 to 56% of the total catch.

Table 1 shows the percentage species composition (weight), as determined from the sampling net and from the total catch, and the percentage error of each species. Chi-square values based on catch weights indicate that the species composition in the sample differed from the total catch

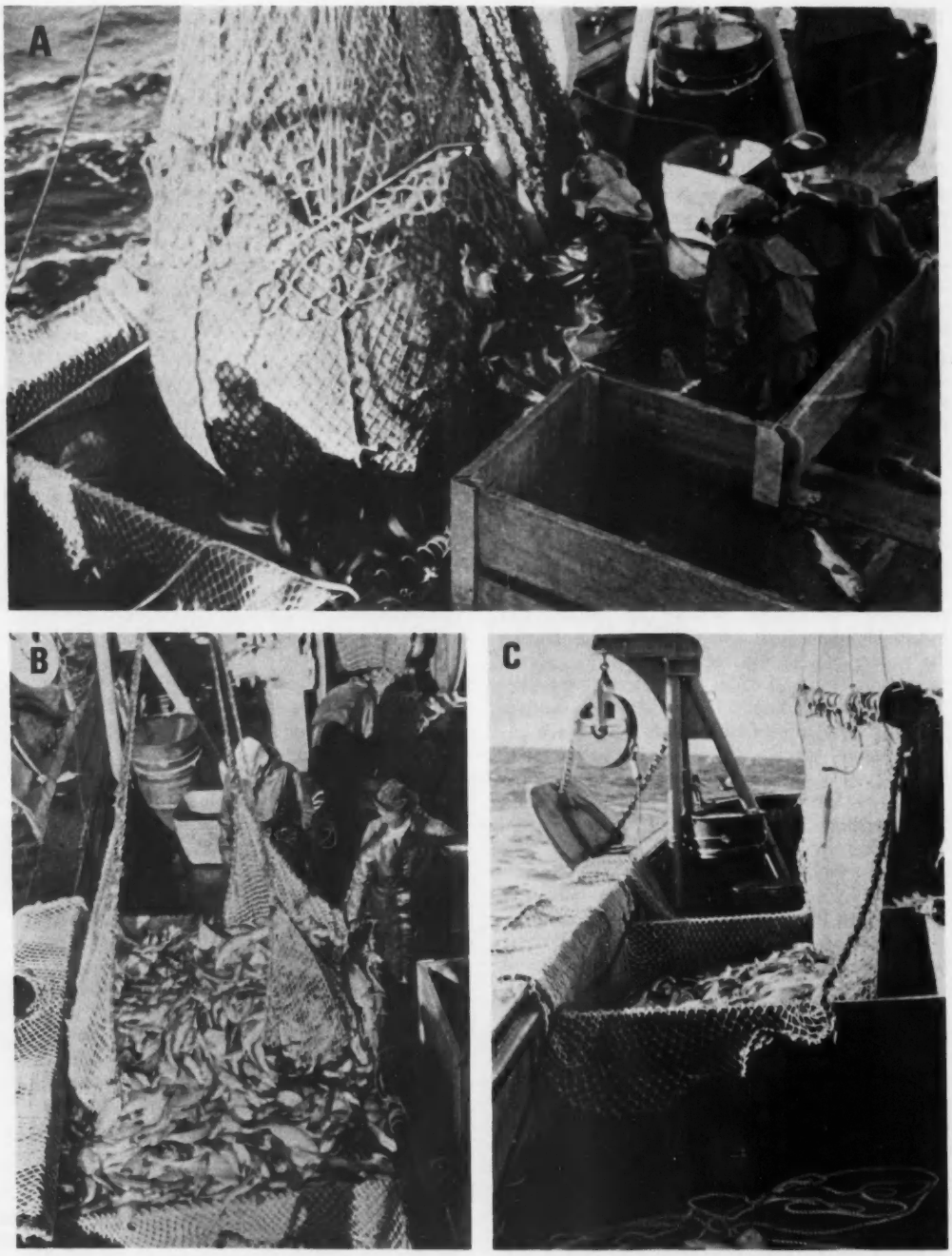

FIG. 1. Sequence of events during mechanical sampling of the catch: weighing, properly orienting the codend, and releasing the catch into deck bin, cargo net, and retainer net; sampling the catch using the cargo net; and rolling unneeded portion of catch overboard with retainer net.

TABLE 1. Percentage species composition in the cargo net (sample) and in the total catch (population), by haul. The percent error in estimating the weight of each species in the population from the sampled portion is indicated, as are chi-square values.

Species	Haul no. 1			Haul no. 2			Haul no. 3			Haul no. 4			Haul no. 5		
	Cargo net sample (%)	Total catch (%)	Error[a] (%)	Cargo net sample (%)	Total catch (%)	Error (%)	Cargo net sample (%)	Total catch (%)	Error (%)	Cargo net sample (%)	Total catch (%)	Error (%)	Cargo net sample (%)	Total catch (%)	Error (%)
English sole	34.0	33.0	+2.9	43.2	44.6	−3.1	42.0	41.1	+2.2	32.1	30.4	+5.6	32.4	29.5	−9.9
Sand sole	15.4	15.9	−3.1	6.3	5.1	+23.8	—	—	—	17.0	15.3	+11.6	18.0	18.1	−0.5
Starry flounder	10.3	9.3	+10.2	—	—	—	13.0	14.2	−8.9	10.6	9.5	+11.1	10.5	11.4	−8.3
Other flatfish	1.6	1.7	−4.8	3.5	4.4	−20.2	6.5	7.0	−6.9	2.2	2.3	+5.9	2.8	2.6	+5.5
Pacific hake	2.3	2.2	+3.8	—	—	—	2.9	2.2	+33.0	—	—	—	—	—	—
Other roundfish	1.4	2.1	−35.3	3.0	3.6	−15.9	0.6	0.6	0.0	11.4	9.3	+22.7	6.9	7.6	−9.4
Pile perch	2.8	3.0	−6.8	—	—	—	—	—	—	5.9	8.6	−32.2	6.3	6.8	−7.0
Copper rockfish	0.9	1.4	−36.4	—	—	—	—	—	—	3.3	4.2	−19.9	12.2	12.6	−2.8
Poachers and sculpins	2.8	2.7	+1.5	2.3	2.5	−10.0	0.7	0.7	0.0	2.9	4.2	−30.8	2.8	3.1	−8.3
Ratfish	—	—	—	—	—	—	3.4	3.7	+9.5	—	—	—	—	—	—
Spiny dogfish	27.3	27.1	+0.6	41.7	39.7	+4.8	32.4	30.4	+6.4	13.9	12.7	+9.4	6.7	7.1	−5.0
Skates	0.9	1.0	−16.0	—	—	—	—	—	—	—	—	—	—	—	—
Dungeness crab	0.5	0.5	+1.2	—	—	—	—	—	—	—	—	—	1.3	1.2	+11.0
Total catch in pounds and (in parentheses) kilograms	4350 (1973)			1530 (694)			1749 (793)			2475 (1123)			1768 (802)		
Fraction in sample	.558			.541			.493			.448			.556		
χ^2	15.36			5.93			5.92			27.33			5.37		
$\chi^2_{0.10}$	17.28			9.24			12.02			13.40			14.68		

[a]Percent error $= \frac{\text{observed sample wt} - \text{expected sample wt}}{\text{expected sample wt}}(100)$.

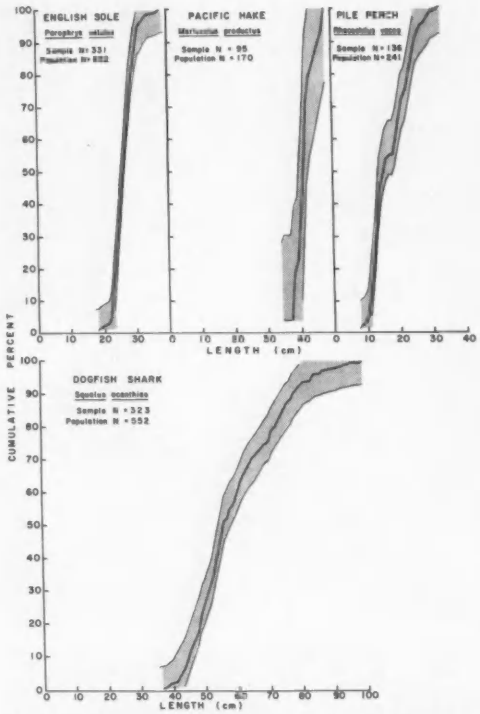

FIG. 2. Comparison of size composition of four species from the cargo net (sample) with the total catch (population). The solid line represents the observed distribution of the sample; and the shaded area, a 90% confidence band plotted around the distribution of the population.

in only one haul (no. 4, Table 1). The percent error associated with species composing at least 20% of catches was always less than 10% and was most often overestimated. Errors greater than 25% were generally associated with species composing less than 5% of the total catch and were most often underestimated.

Further empirical tests should be conducted to determine if cargo net samples of less than 45% of the total catch are representative. Although the need for such information rarely occurred during regular assessment surveys where 30-min tows seldom exceeded 3600 kg, recent production fishing on board the charter vessel *Anna Marie* resulted in many catches in excess of 6000 kg which raises some concern about the ability of cargo net samples to represent the catch composition. In such instances, the total catch was placed in a large fish bin, split by split and the cargo net set to remove about a 1200 kg sample as usual. This was accomplished by reducing the spread of the cargo net, so that for example only 20% was removed for processing. While it did not appear that sampled portions that were less than 45% of the total catch were biased, it will be important to investigate this possibility in the future.

Length data were collected and analyzed from English sole, *Parophrys vetulus*; sand sole, *Psettichthys melanostictus*; starry flounder, *Platichthys stellatus*; pile perch, *Rhacochilus vacca*; copper rockfish, *Sebastes caurinus*; Pacific hake, *Merluccius productus*; spiny dogfish, *Squalus acanthias*; and ratfish, *Hydrolagus colliei* to determine if the size of fish in the mechanically sampled portion was representative of the total catch. A total of 8354 fish in 23 sets of length data were measured and compared.

Direct comparison of lengths from the total catch (population)[1] with lengths in the cargo net (sample) were made. Each of the 23 data sets showed that the distribution of lengths in the sample were representative of their respective populations since the sample frequencies did not deviate from the population limits. The four examples shown in Fig. 2 are typical of results obtained from flatfish, roundfish, rockfish, and elasmobranchs. As discussed by Dixon and Massey (1969), the band represents the maximum we can expect (90% of the time) the sample cumulative distribution to deviate from the cumulative distribution of the population under the hypothesis that the sample was drawn at random from the total catch.

Conclusions — Tests conducted in Puget Sound, suggest that cargo net samples (of 45–55% of the total catch) adequately represent the species composition and size composition of species in the total catch. Although species diversity sampled in Puget Sound is not as great as in the Gulf of Alaska survey areas, representatives of the major fish groups present in the Gulf were sampled and included in the analysis. Results support the assumption that the codend fills in a bilaterally symmetrical fashion; however, they do not disprove the possibility of radial symmetry. Although I doubt that radial symmetry exists, primarily because of the general movement of fish with closed air bladders toward the dorsal side of the codend during retrieval, this possibility could be tested by mechanically sampling the catch after rotating the codend 90° out of phase.

[1]Population refers to a statistical population from which the samples were drawn.

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Growth and Survival of Young-Of-The-Year Emerald Shiners (*Notropis atherinoides*) at Different Temperatures

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MCCORMICK, J. H., AND C. F. KLEINER. 1976. Growth and survival of young-of-the-year emerald shiners (*Notropis atherinoides*) at different temperatures. J. Fish. Res. Board Can. 33: 839–842.

Young-of-the-year emerald shiners (*Notropis atherinoides*) were exposed to mean constant temperatures of 6.9, 11.9, 16.0, 19.8, 24.0, 26.9, 28.9, 31.0, 32.8, 34.9, and 36.7 C for 6 wk. Maximum rates of growth and net biomass gain occurred at 28.9 C, but these rates were not statistically greater ($P > 0.05$) than those at 26.9 and 24.0 C. Death rates were low at test temperatures up to 32.8 C but high at 34.9 C and above. The upper incipient 7-day TL50 was estimated to be 35.2. The 1-day TL50 of fish taken from the natural habitat, where peak daily temperatures ranged between 20 and 25 C during the preceding few days, was 32.6 C. Natural production of this important forage species would probably be best where temperatures are at least 19 C but not above 29 C for extended periods.

MCCORMICK, J. H., AND C. F. KLEINER. 1976. Growth and survival of young-of-the-year emerald shiners (*Notropis atherinoides*) at different temperatures. J. Fish. Res. Board Can. 33: 839–842.

Les auteurs ont exposé des jeunes ménés émeraude (*Notropis atherinoides*) de l'année aux températures constantes moyennes de 6.6, 11.9, 16.0, 19.8, 24.0, 26.9, 28.9, 31.0, 32.8, 34.9 et 36.7 C durant 6 semaines. Le rythme de croissance et le gain net de biomasse maximums se produisent à 28.9 C, mais ne sont pas significativement plus grands ($P > 0.05$) qu'à 26.9 et 24.0 C. La mortalité est faible à des températures d'essai allant jusqu'à 32.8 C, mais est élevée à 34.9 C et plus. On estime à 35.2 la LT50 supérieure du début des mortalités après 7 jours. La LT50 après 1 jour de poissons retirés de leur habitat naturel, avec des températures journalières de pointe allant de 20 à 25 C durant les quelques jours précédents, est de 32.6 C. La production naturelle de cet important poisson fourrage serait probablement optimale là où la température est d'au moins 19 C mais non au-delà de 29 C sur des périodes prolongées.

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THE emerald shiner (*Notropis atherinoides*) is an important forage fish in the Great Lakes and many other large inland waters (Cooper 1936; Flittner 1964; Walburg 1964; Campbell and MacCrimmon 1970). Because of its preference for large bodies of water, and the similar site preferences of industries requiring large quantities of cooling water with related heated discharges, this species is destined to encounter thermally altered habitats.

Some aspects of their thermal relations are

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TABLE 1. Responses of young-of-the-year emerald shiners (*Notropis atherinoides*) exposed 6 wk to constant temperatures while receiving unrestricted ration. Instantaneous rates are pooled means of three, 10-fish subsamples, one from each replicate for three sampling periods of 2 wk each. Weights at the start and end are means from three, 10-fish subsamples, one from each replicate.

Temperature (C) (mean and range)[a]	Wt (g)		Instantaneous rates (%/day)		
	At start	At end of 6 wk	Growth	Mortality	Net biomass gain[b]
6.9 (6.0–7.6)	0.06	0.08	0.66	0.04	0.62
11.9 (11.1–13.1)	0.06	0.09	0.83	0.12	0.71
16.0 (15.9–16.3)	0.07	0.16	1.97	0.02	1.95
19.8 (18.1–20.0)	0.12	0.41	2.92	0.01	2.91
24.0 (18.9–24.2)	0.16	0.80	3.86	0.00	3.86
26.9 (21.2–27.1)	0.19	0.95	3.80	0.00	3.80
28.9 (21.3–29.5)	0.19	1.05	4.10	0.00	4.10
31.0 (25.3–31.4)	0.24	0.84	3.00	0.00	3.00
32.8 (25.4–33.5)	0.22	0.64	2.63	0.11	2.52
34.9 (28.2–35.5)	0.20	—[c]	−0.40[d]	4.10[d]	−4.50[d]
36.7 (35.4–36.9)	0.14	—[e]	—[e]	28.66[e]	—[e]

[a]The 95% confidence limits of all reported mean temperatures is <0.1 C.
[b]Least significant difference, 95% C.L. = 0.91%/day.
[c]All fish died before 6 wk of exposure had been completed.
[d]Based on first 2 wk only. Too few fish remained for testing beyond the first sampling period.
[e]All dead within 1st wk.

already known. Spawning begins as the water reaches 20–22 C and continues through 27 C usually May through August (Gray 1942; Flittner 1964; Campbell and MacCrimmon 1970). Preferred temperatures, at summer acclimations, have been reported as high as 27 C from field observations (WAPORA 1971) and 22–25 C from laboratory studies (Campbell and MacCrimmon 1970; Barans and Tubb 1973). Barans (1972) showed that adults avoided temperatures above 27 C, and young-of-the-year above 30 C. Hart (1947) studied incipient lethal temperatures for the emerald shiner over a series of acclimation temperatures and found 30.7 C to be the highest temperature tolerated for 7 days. This study sought information about effects of temperature on the growth and survival of emerald shiners (*N. atherinoides*) during their first growing season.

Materials and Methods — The test system provided 225 ml/min of untreated Lake Superior water at constant temperatures to triplicate glass test chambers each 10 × 10 × 40.5 cm long (McCormick and Syrett 1970 unpublished data). Temperatures were monitored with a recording telethermometer calibrated once daily with a standardized mercury thermometer. The temperatures reported are the means of the daily thermometer readings except when temperature-control failed. When deviations exceeded 1 C for 1 h or more, a daily mean was calculated from the thermograph. Treatment temperatures reported are the pooled mean of three replicates. The 95% confidence limits about the reported means were all within ± 0.1 C, even though temperature-control failures permitted temperatures ranging beyond this narrow margin (Table 1). The magnitude and duration of failures were not considered sufficient to influence the results attributed to the reported treatment temperatures.

Young-of-the-year emerald shiners, approximately 15 mm long and 0.03 g in weight were collected from St. Louis Bay at the western end of Lake Superior on August 17, 1970. The water temperature was 19 C; temperatures from the same area 1 wk earlier were up to 23 C. After 13 days at 20 C the fish were transferred in a stratified random manner to their respective test chambers, 70 fish per chamber. Three extra chambers added every seventh stratum provided three, 10-fish samples for initial size determination. Over the next 18 days temperatures were adjusted from 20 C at the rate of 1 C/day to test temperatures (Table 1) to insure acclimation (Brett 1944).

Characteristics of the test water have been described by Biesinger and Christensen (1972). Dissolved oxygen concentrations during the experiment were all 5 ppm or higher with a mean saturation of 88% (68–99%). Fluorescent lamps gave a mean 12-h photoperiod normal for September–October at the Duluth, Minn. latitude, approximately 47° N.

The ration, newly hatched brine shrimp, was added in early morning, later afternoon, and during the day whenever it was noticed that most of the food was gone. On weekends fish were fed only once or twice daily.

Test chambers were cleaned daily, and dead fish were counted and removed. Subsamples of 10 fish/replicate were removed every 2 wk for weight measurements. Samples were taken in the morning before feeding so that little, if any, of the weight would be unassimilated food (Voightlander and Wissing 1974).

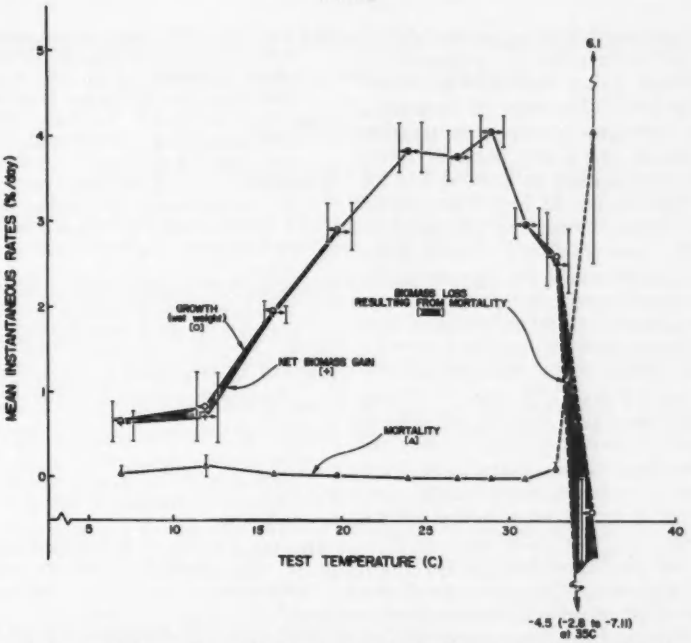

FIG. 1. Effects of temperature on mean instantaneous mortality and rates of growth and net biomass gain of young-of-the-year emerald shiners (*Notropis atherinoides*) during 6 wk of growth with excess ration. Vertical bars set to the side of the data points indicate the range of the replicate means. Dashed lines indicate data obtained from only the first 2 wk of exposure.

The sampled fish were preserved in formalin–picric–trichloroacetic solution (Davenport 1960) then later removed, blotted, and weighed intact to the nearest 0.1 mg. Ten additional fish were removed from each chamber at the end of the 2nd, 4th, and 6th wk for other experimental purposes[1] and to adjust population density as the individuals increased in size.

Instantaneous rates of mortality, growth, and net biomass gain were calculated for each sampling interval on a percentage per day basis (Ricker 1958). The rate of net biomass gain was derived by subtracting weight lost because of deaths within a test population from the rate of weight gain by survivors within the same population. Analysis of covariance was used to mathematically reduce the mean size of fish at all test temperatures to a common initial size, since size equivalency had been lost (Table 1) during the 18-day acclimation period before the start of the exposure period. The weighted mean rates for the three sampling periods were used as treatment effects in the analysis. The least significant difference (L.S.D.) derived from this calculation established the effect of temperature on the rate of net biomass gain considered significant at the 95% level. TL50 values were calculated by the method of Litchfield and Wilcoxon (1949).

Results and discussion — Mortalities were negligible, 0.00–0.12%/day, at temperatures from 6.9 to 32.8 C (Table 1, Fig. 1); no fish died from 24.0 to 31.0 C. At 34.9 C the death rate increased sharply to 4.10%/day; at 36.7 C all fish were dead within the first week. Rates of net biomass gain and rates of growth were similar at 6.9–19.8 and 32.8 C and were statistically equal at 24.0–31.0 C. The high mortality rate at 34.9 C makes this temperature as unsuitable for young emerald shiners. Mean instantaneous rates of net biomass gain were maximum (3.80–4.10%/day) at 24.0 through 28.9 C.

Growth at 31.0 C was comparable to that at 19.8 C; we suggest, however, that it may be less desirable as it lies on the too warm side of the temperature range promoting maximum growth, indicating that growth retardation is due to heat

[1]To examine the suitability of brine shrimp as a diet for fish (Bardach et al. 1972), samples of fish (27 fish after 2 wk of testing and 30 fish at test termination, 4 wk later) were analyzed by gas chromatography for total DDT analogue residues. Both groups had concentrations below 1 ppm, well within the no-known-effect level for fish. (A. W. Jarvinen, and L. H. Mueller personal communication 1974, Pesticide Study Group, National Water Quality Laboratory, Duluth, Minn.)

stress. Further evidence that temperatures of 31.0 C and higher are undesirable for young-of-the-year emerald shiners is that they avoid such temperatures (Barans 1972). The onset of teratogenic effects, such as curvatures of the spine observed in one or two fish, at 32.8 C and reduced growth rates marks this temperature as questionable for this life stage of the species. At 34.9 C teratogenic effects similar to those depicted by Tsukuda and Katayama (1957) were observed in several specimens and were accompanied by high mortality rates and slow growth resulting in negative rates of net biomass gain, clearly identifying this temperature as unsuitably high for these fish.

The 1st wk's mortality data provided an ultimate upper incipient 7-day TL50 value of 35.2 C. At 36.5 C slightly more than half the fish died in the first 3–4 days of exposure. Hart (1947) reported 30.7 C as the incipient 7-day TL50 for a mixed age-group of emerald shiners (84% age-group 0, 16% age-group I) when acclimated to 25 C. Our results show that acclimation to 25 C is not adequate to acquire tolerance to the 7-day ultimate upper incipient lethal limit which we suggest to be nearer 35 C. With fish taken directly from St. Louis Bay (ambient temperature 19 C), a 24-h TL50 value of 23.6 C was found, a result comparable to Hart's (1947) finding. The temperature history of these fish was unknown, but maximum daily temperatures on the preceding days, and thus their acclimation, was not greater than 20–25 C.

As in all growth rate studies in which an abundant to unlimited ration is used, it must be emphasized that in the wild, food is more often a limiting factor. Accordingly, maximum rates of net biomass gain would be expected to occur at progressively lower temperatures as food becomes more limiting (Brett et al. 1969).

Thus, for the well-being of young-of-the-year emerald shiners and consequently the well-being of those more frequently sought-after species that use them as food, the combined results suggest that 29 C is the maximum temperature expected to result in most rapid net biomass gain, and when food is limiting, depending on how limiting, some lower temperature would be best. During the growing season, however, temperatures below 15 C are also undesirable, and in such instances adding heat to their habitat when properly controlled may be beneficial both in extending the growing season and in stimulating daily forage production rates.

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Mixed Species Schooling of Postlarvae of *Ammodytes hexapterus* and *Clupea harengus harengus*

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RICHARDS, S. W. 1976. Mixed species schooling of postlarvae of *Ammodytes hexapterus* and *Clupea harengus harengus*. J. Fish. Res. Board Can. 33: 843–844.

Small schools of post-larval *Ammodytes hexapterus* and *Clupea harengus harengus* were observed and captured at Margaree Harbor entrance in the Gulf of St. Lawrence on July 20, 1974 and captured at Goldsmith Inlet beach in Long Island Sound on April 18, 1975. These species appeared similar, yet *A. hexapterus* averaged 14 mm longer than *C. harengus harengus* at Margaree, but 9 mm shorter than herring at Goldsmith Inlet beach. In both areas sand lances were narrower than herring.

Post-larval *A. hexapterus* do not bury in the sand, and they will school together with post-larval *C. harengus harengus* regardless of the differences in sizes between the two species.

RICHARDS, S. W. 1976. Mixed species schooling of postlarvae of *Ammodytes hexapterus* and *Clupea harengus harengus*. J. Fish. Res. Board Can. 33: 843–844.

De petits bancs d'*Ammodytes hexapterus* et de *Clupea harengus harengus* postlarvaires ont été vus et capturés à l'entrée de Margaree Harbour, dans le golfe Saint-Laurent, le 20 juillet 1974 et capturés sur la plage de Goldsmith Inlet, dans le détroit de Long Island, le 18 avril 1975. Ces espèces ont la même apparence; cependant, *A. hexapterus* est en moyenne 14 mm plus long que *C. harengus harengus* à Margaree, mais 9 mm plus court que le hareng sur la plage de Goldsmith Inlet. Aux deux endroits, le lançon est plus étroit que le hareng.

Les contenus stomacaux indiquent que les deux espèces se nourrissent des mêmes proies. Bien qu'il y ait chevauchement dans le choix de la nourriture, *A. hexapterus* mange plus de gros copépodes que *C. harengus*.

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Mixed species schooling of postlarvae of Ammodytes hexapterus and Clupea harengus — Although apparently rarely observed, mixed species schooling is known for various species of fish (Barlow 1974; Breder 1959; Radakov 1973) including the Ammodytidae (Kühlmann and Karst 1967). The latter study described mixed schools of species of two sand lances, *Ammodytes marinus* and *Hyperoplus lanceolatus* in the Baltic Sea. Adults of both species, of approximately the same size, formed schools inshore to travel to and from their feeding grounds offshore.

This report describes mixed species schooling of postlarvae of Pacific sand lance, *Ammodytes hexapterus*, with the postlarvae of Atlantic herring, *Clupea harengus harengus*, from two localities on the east coast of North America.

Methods — *Ammodytes hexapterus* postlarvae and postlarvae of *Clupea harengus harengus* were observed in the Gulf of St. Lawrence in midafternoon of July 20, 1974, schooling together along the beach to the north of the breakwater at the entrance of Margaree Harbor, Cape Breton Island. On April 18, 1975, at 0930, mixed schools of post-larval *A. hexapterus* and *C. harengus* were caught in Long Island Sound along the beach outside of Goldsmith Inlet on the north shore of Long Island. Although the two species look alike, they can be separated in the field by the general shape. *Clupea harengus harengus* is deeper-bodied than *A. hexapterus* of similar lengths.

Specimens from Margaree Beach were captured in a 6-m beach seine of 5-mm bar mesh; specimens from Goldsmith Inlet beach were captured in a model of a Danish sandeel trawl, 9 m wide at the mouth, of 10-mm bar mesh with a 5-mm bar mesh liner in the cod end. They were preserved in 10% buffered formalin. Standard lengths were measured to the nearest mm.

Results — Observations along the beach at Margaree were made while snorkeling. The schools were small, approximately 1–1.3 m long, 0.6 m wide, and 0.5 m deep. They were wedge-shaped ("travelling schools" of Radakov 1973) consisting of 200–500 postlarvae of similar appearance.

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TABLE 1. Summary of standard lengths and depths of *Ammodytes hexapterus* and *Clupea harengus harengus* postlarvae from Margaree Beach (Gulf of St. Lawrence) and Goldsmith Inlet Beach (Long Island Sound).

	Ammodytes hexapterus		*Clupea harengus harengus*	
	Margaree	Goldsmith	Margaree	Goldsmith
No. fish measured	20	11	20	20
Range and (mean) of standard length	32–51(45)	17–33(24)	25–37(31)	31–35(33)
Range and (mean) of depth	4–6(4.9)	1.5–2.1(1.8)	4–6(5.4)	4.4–5.7(5.0)

Close examination showed that there were only slightly more herring than sand lances, and that the lances were distributed throughout the whole school. We chased the schools, noting that the sand lances made no attempt to escape by burying in the sand, but the whole school of both species swam away or hid behind the tentacles of a red jellyfish, *Cyanea* sp., abundant along the beach at this time.

Schools of post-larval sand lances only, of approximately the same length (30–50 mm), were observed and chased at the Magdalen Islands and at St. Ann's Bay, Cape Breton Island. They also swam away; no attempt was made to bury in the sand. On July 8, 1974 at Conway Inlet, Malpeque Bay, Prince Edward Island, I chased a single post-larval sand lance of approximately 50 mm for 10 min. No attempt was made by the isolated lance to bury in the sand. Instead, it kept swimming against the tidal current, just out of reach below or in front of the observer.

Normally, fish in schools are of similar size (Shaw 1962). Post-larval sand lances from Margaree averaged 45 mm or 14 mm longer than the post-larval herring at 31 mm, but were thinner than the herring (Table 1). Post-larval sand lances from Goldsmith Inlet beach were shorter than those from Margaree, averaging 24 mm, and were shorter than the herring with which they were schooling. The herring, there, were similar in length to those from Margaree.

Apparently these two species will school together during their first year, regardless of their differences in total lengths and widths.

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Protection of Steelhead Trout (*Salmo gairdneri*) Against Vibriosis with a Living Low-Virulence Strain of *Vibrio anguillarum*

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BRAATEN, B. A., AND H. O. HODGINS. 1976. Protection of steelhead trout (*Salmo gairdneri*) against vibriosis with a living low-virulence strain of *Vibrio anguillarum*. J. Fish. Res. Board Can. 33: 845–847.

Exposure to a living low-virulence strain of *Vibrio anguillarum* was found to protect steelhead trout (*Salmo gairdneri*) against a subsequent challenge of highly virulent *V. anguillarum*. The fish were effectively protected by means of a single intraperitoneal injection or by multiple oral vaccinations.

BRAATEN, B. A., AND H. O. HODGINS. 1976. Protection of steelhead trout (*Salmo gairdneri*) against vibriosis with a living low-virulence strain of *Vibrio anguillarum*. J. Fish. Res. Board Can. 33: 845–847.

L'exposition de truites steelhead (*Salmo garidneri*) à une lignée vivante de faible virulence de *Vibrio anguillarum* protège les poissons contre une attaque subséquente de *V. anguillarum* de forte virulence. Une seule injection intrapéritonéale ou de multiples vaccinations orales donnent aux poissons une protection effective.

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Vibrio anguillarum is indigenous to the marine environment and is one of the most lethal of fish pathogens, causing vibriosis. In pen-reared salmonids in Puget Sound, Washington, vibriosis can occur during all seasons of the year, but outbreaks are more frequent and severe during the summer and early fall months. The disease can be partially controlled by oral administration of antibacterial drugs; killed oral and parenteral vaccines have also been used with varying degrees of success (Hayashi et al. 1964; Fryer et al. 1972; Gunnels 1974). In our laboratory it was observed, upon testing the virulence of several strains of *V. anguillarum* for anadromous *Salmo gairdneri* (steelhead trout), that one isolate was much less virulent than other vibrios isolated from diseased salmonids. Furthermore, fish injected with this isolate were found to be protected against a subsequent intraperitoneal (IP) injection of virulent *V. anguillarum*. This note is a report of preliminary work on protecting fish against vibriosis using live *V. anguillarum* vaccines.

Materials and methods — Steelhead trout were obtained from the National Fish Hatchery, Quilcene, Washington, and held in fiberglass tanks supplied with fresh water at a temperature of 15–16 C. The fish were fed Oregon moist pellets. For experimentation the fish were moved to fiberglass tanks in an isolation laboratory. The water temperature in these tanks was 17–18 C and the fish were allowed to adapt for 72 h before any experimental work was begun. The average weight of the fish was 42 g with a range of 18–94 g.

Several strains of *V. anguillarum* were generously supplied by A. J. Ross of the Western Fish Disease Laboratory, U.S. Fish and Wildlife Service, Seattle, Washington. A number of biochemical tests were run on each strain of the bacterium and results were compared to those of the archetype of *V. anguillarum* described by Evelyn (1971). Each strain was also tested for its pathogenicity to steelhead trout. The bacteria were maintained under paraffin oil at room temperature on slants of trypticase soy agar. For in vivo experimentation, the bacteria were grown for 24 h at room temperature in trypticase soy broth (TSB). Both the slants and broth contained an additional 1% NaCl. In the parenteral immunization experiments, each fish was given 0.1 ml IP of a 24-h culture of living low-virulence *V. anguillarum*. Each control fish received 0.1 ml IP of sterile TSB. The fish were held at 17–18 C for 2 wk before they were challenged IP with 0.1 ml of a 1:1000 dilution of a 24-h culture of virulent *V. anguillarum*.

Oral administration of the living low-virulence *V. anguillarum* strain was done as follows. The pellets

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TABLE 1. Comparison of mortality due to vibriosis within 72 h in parenterally vaccinated fish vs. control fish. Fish had been injected IP with low-virulence *Vibrio anguillarum* or sterile medium 2 wk before an IP challenge with virulent *V. anguillarum*.

Days after start of expt		
0	14	17
Fish injected IP with	No. of virulent bacteria in IP challenge dose per fish	No. fish dead / No. challenged
5×10^8 low-virulence bacteria in trypticase soy broth	1×10^6	0/15
Trypticase soy broth only	1×10^6	14/15

TABLE 2. Comparison of mortality due to vibroisis within 72 h in orally vaccinated fish vs. controls. Test fish had been given multiple administrations of food containing low-virulence *V. anguillarum* before receiving an IP challenge of virulent *V. anguillarum*. Control fish received multiple oral doses of food treated with sterile fresh bacterial growth medium only.

	Days after start of expt	
	14	17
Fish fed dry pellets soaked with[a]	No. of virulent bacteria in IP challenge dose per fish	No. fish dead / No. challenged
Low-virulence bacteria in trypticase soy broth	1×10^6	2/30
Trypticase soy broth only	1×10^5	14/14

[a]Fed on days 0, 2, 4, 6, 8, 10, and 12.

of a dry feed, Purina Trout Chow Developer,[2] were mechanically cracked (so that they would not float) and added to a 24-h TSB culture of the low-virulence strain. The food was soaked for 15 min at each feeding and fed to the fish to satiation. The fish were fed every other day for 7 times and challenged IP 2 days after the last feeding. Control fish received feed soaked in sterile TSB. In all experiments, the number of viable virulent bacteria injected IP was determined by plate count.

Results and discussion — The data in Table 1 show that the low-virulence *V. anguillarum* strain was effective in protecting against the virulent strain ($P = 0.01$). In this experiment all control fish that were killed were dead within 72 h after being injected IP with the virulent strain. *Vibrio anguillarum* was later isolated from the kidneys of these fish. None of the challenged parenterally vaccinated fish died; they all appeared healthy over an observation period of 3 mo.

It is clear from the results shown in Table 2 that multiple oral feedings also provided a high degree of protection ($P = 0.01$). We found that even a single oral feeding of live vaccine appeared to confer some protection.

In subsequent laboratory trials we have not demonstrated that better protection is induced by the live-bacteria vaccines than by killed-bacteria vaccines. These results are not definitive, however.

[2]Mention of product names in this paper does not imply endorsement by the National Marine Fisheries Service.

Live *Vibrio* vaccines may well be more effective than killed *Vibrio* vaccines under conditions of natural exposure to vibriosis because of a better ability to reach appropriate sites for induction of an immune response, or because of less alteration of bacterial antigens. Before live *Vibrio* vaccines are tested at saltwater sites questions of potential hazard must be further evaluated.

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Developmental Stages of *Pontella meadi* Wheeler (Copepoda: Calanoida)[1]

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GIBSON, V. R., AND G. D. GRICE. 1976. Developmental stages of *Pontella meadi* Wheeler (Copepoda: Calanoida). J. Fish. Res. Board Can. 33: 847–854.

The complete development of *Pontella meadi* is described and illustrated on the basis of nauplii and copepodids cultured in the laboratory and collected in the field. Development from nauplius to adult took from 18–25 days at 20 C when animals were fed dinoflagellates and *Artemia* nauplii.

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Nous décrivons et figurons le développement entier de *Pontella meadi* à partir de nauplii et de copépodites élevés en laboratoire. Le développement du stade nauplius à l'adulte requiert de 18–25 jours à 20 C quand les animaux sont nourris des dinoflagellés et de nauplii d'*Artemia*.

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Pontella meadi Wheeler is a large predatory copepod that occurs in neritic waters along the Atlantic coast from east of Cape Cod to Florida, and in the Gulf of Mexico from the Florida Keys to Texas. It is an inhabitant of the neuston and may be quite common in surface plankton collections. Since few species of the family Pontellidae have been cultured in the laboratory, the developmental stages are poorly known.

Materials and methods — The original description of *P. meadi* was based on immature female and adult male specimens collected in Woods Hole Harbor, Massachusetts (Wheeler 1900). Subsequently, Wilson (1932) described (as *P. pennata*) specimens that were obtained from Chesapeake Bay. Fleminger (1957) suggested that *P. pennata* is conspecific with *P. meadi*. Earlier he (Fleminger 1956) recognized two sympatric forms of adult male *P. meadi* (termed high and low dimorphs) that are separated by size and certain morphological characters. The males described by Wilson are considered high dimorphs. Both morphological types have been observed in our collections from near Woods Hole.

A neuston net (mesh size 243 μm) was used to collect live *P. meadi* from the surface waters of Vineyard Sound (41°32′N, 70°45′W) during August 1974. Adult females were isolated in dishes (125 cm^3) containing filtered sea water. Eggs were usually laid within 24 h and hatched in about 2 days at 18–20 C. Nauplii were fed dinoflagellates, especially *Prorocentrum micans*, *Gymnodinium nelsoni*, and *Gonyaulax polyedra*, and copepodids were fed *Artemia* nauplii and *Gymnodinium*. Representatives of each developmental stage were preserved in 8%

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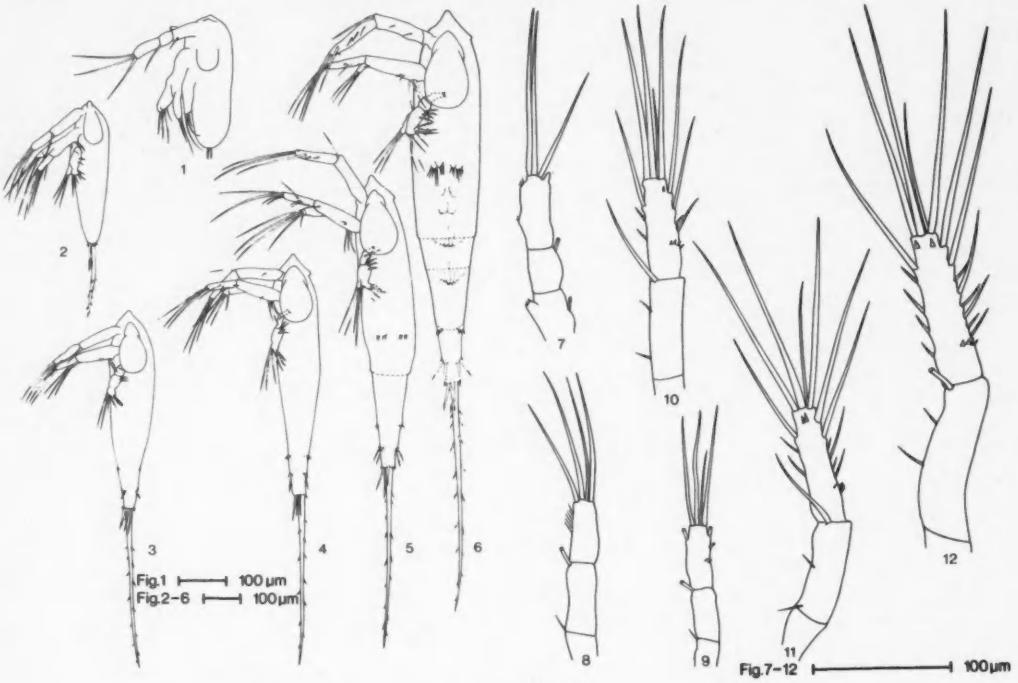
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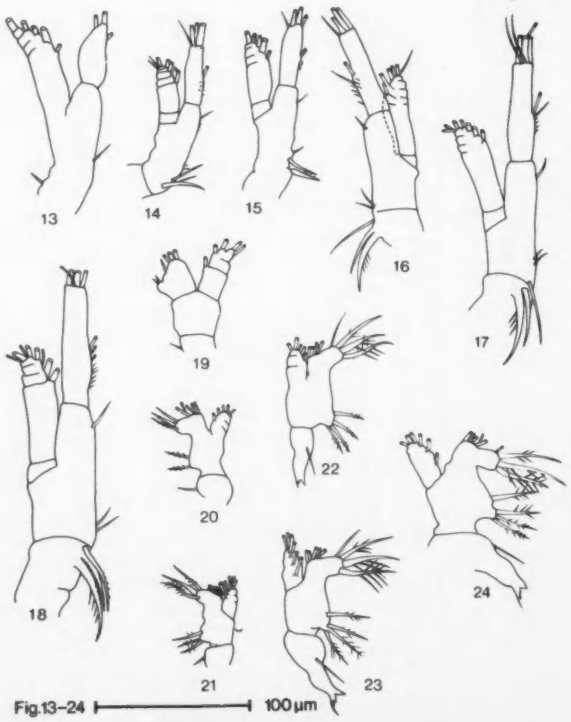
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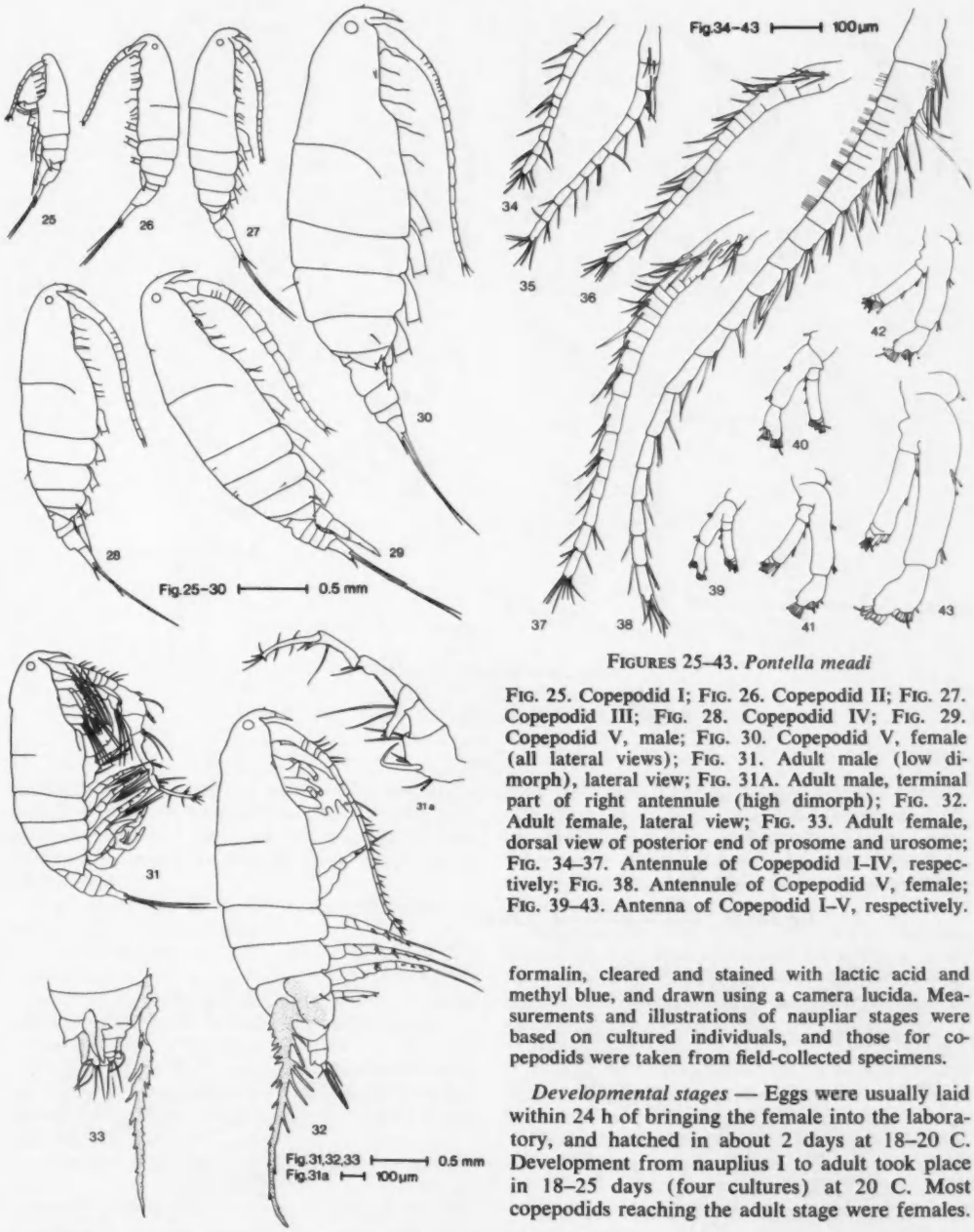


FIGURES 25–43. *Pontella meadi*

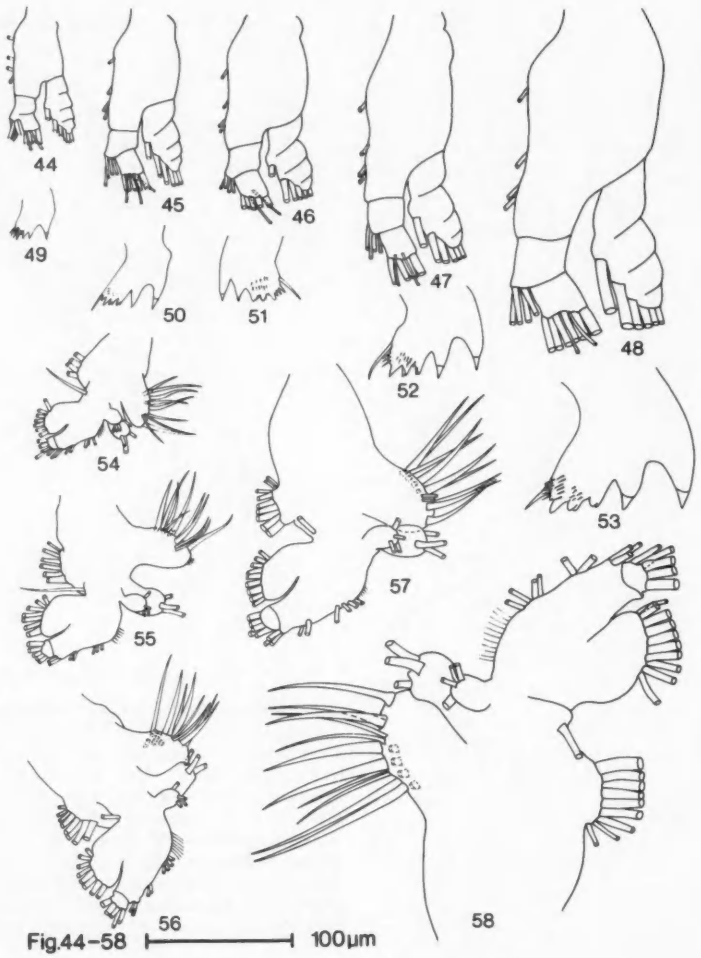
FIG. 25. Copepodid I; FIG. 26. Copepodid II; FIG. 27. Copepodid III; FIG. 28. Copepodid IV; FIG. 29. Copepodid V, male; FIG. 30. Copepodid V, female (all lateral views); FIG. 31. Adult male (low dimorph), lateral view; FIG. 31A. Adult male, terminal part of right antennule (high dimorph); FIG. 32. Adult female, lateral view; FIG. 33. Adult female, dorsal view of posterior end of prosome and urosome; FIG. 34–37. Antennule of Copepodid I–IV, respectively; FIG. 38. Antennule of Copepodid V, female; FIG. 39–43. Antenna of Copepodid I–V, respectively.

formalin, cleared and stained with lactic acid and methyl blue, and drawn using a camera lucida. Measurements and illustrations of naupliar stages were based on cultured individuals, and those for copepodids were taken from field-collected specimens.

Developmental stages — Eggs were usually laid within 24 h of bringing the female into the laboratory, and hatched in about 2 days at 18–20 C. Development from nauplius I to adult took place in 18–25 days (four cultures) at 20 C. Most copepodids reaching the adult stage were females.

FIGURES 1–24. *Pontella meadi*

FIG. 1. Nauplius I; FIG. 2. Nauplius II; FIG. 3. Nauplius III; FIG. 4. Nauplius IV; FIG. 5. Nauplius V; FIG. 6. Nauplius VI (all ventral views); FIG. 7–12. Antennule of Nauplius I–VI, respectively; FIG. 13–18. Antenna of Nauplius I–VI, respectively; FIG. 19–24. Mandible of Nauplius I–VI, respectively.



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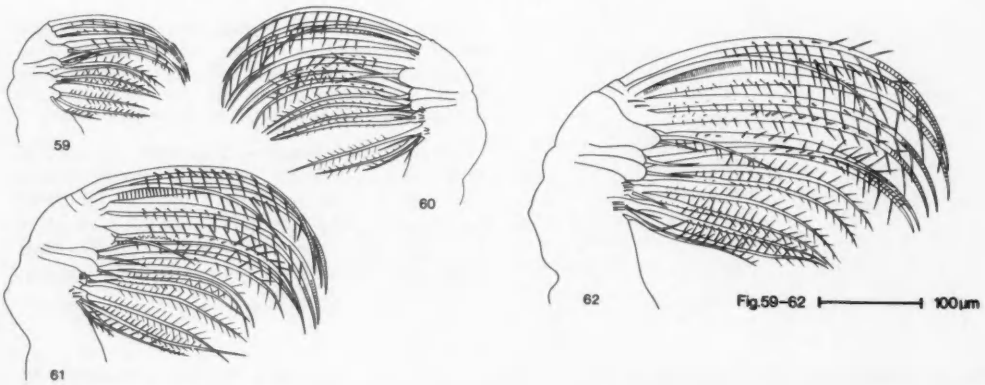
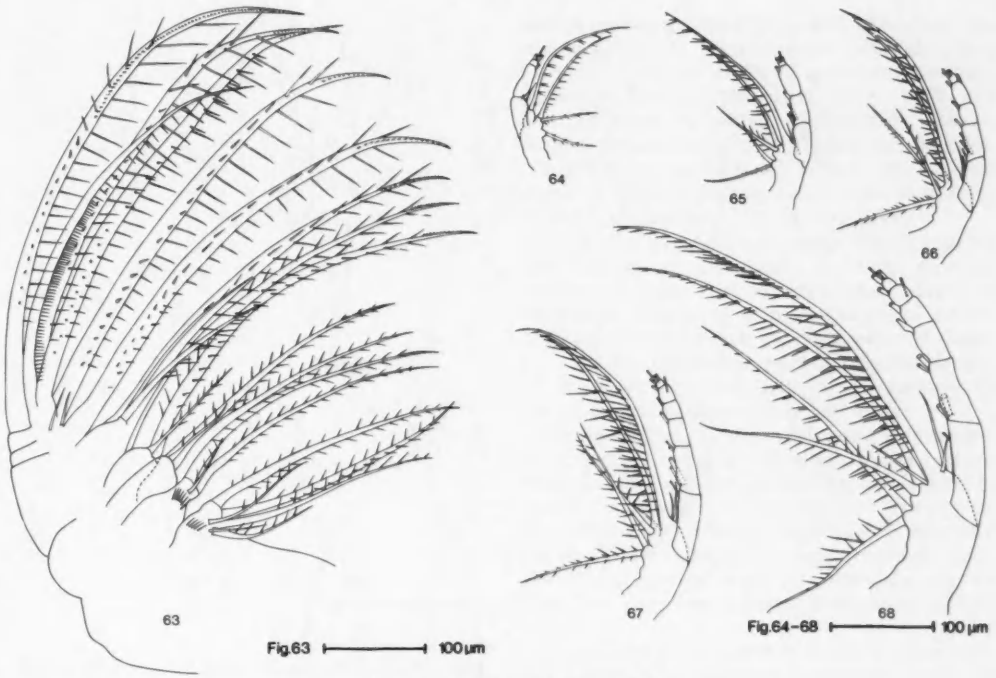


Fig.59–62



FIGURES 64–68. *Pontella meadi*

FIG. 64–68. Maxilliped of Copepodid I–V, respectively.

Late copepodid stages and adults consumed large numbers of newly hatched (less than 1 day old) *Artemia* nauplii. A single adult, for example, may consume as many as 100 or more nauplii in a 24-h period.

Egg — 150 μm in diameter. Reddish-brown in color, with numerous spines.

Nauplius I (Fig. 1, 7, 13, 19) — Length 0.22–0.24 mm. Body oval. Labrum not fully developed. Antennule 3-segmented with three setae on distal segment. Two short, symmetrical caudal spines.

Nauplius II (Fig. 2, 8, 14, 20) — Length 0.37–0.39 mm. Body elongate. Antennule with four setae on distal segment. Two subequal caudal spines: left robust, ½ length of body; right shorter and weaker.

Nauplius III (Fig. 3, 9, 15, 21) — Length 0.46–0.50 mm. Labrum fully developed. Antennule with seven setae on distal segment. Left caudal spine slightly shorter than body, right spine much shorter. Two caudal setae present in this and subsequent stages. One pair of postero-ventral spines near end of body.

Nauplius IV (Fig. 4, 10, 16, 22) — Length 0.58–0.66 mm. Antennule with 11 terminal setae on distal segment. Mandibular blade present. Caudal and postero-ventral spines same as previous stage.

Nauplius V (Fig. 5, 11, 17, 23) — Length 0.76–0.82 mm. Antennule with 13 setae on distal segment. Maxillule represented by several short setae. Two pairs of postero-ventral spines.

Nauplius VI (Fig. 6, 12, 18, 24) — Length 0.86–0.96 mm. Antennule with 17 setae on distal

FIGURES 44–63. *Pontella meadi*

FIG. 44–48. Mandible of Copepodid I–V, respectively; FIG. 49–53. Mandibular blade of Copepodid I–V, respectively; FIG. 54–58. Maxillule of Copepodid I–V, respectively; FIG. 59–63. Maxilla of Copepodid I–V, respectively.

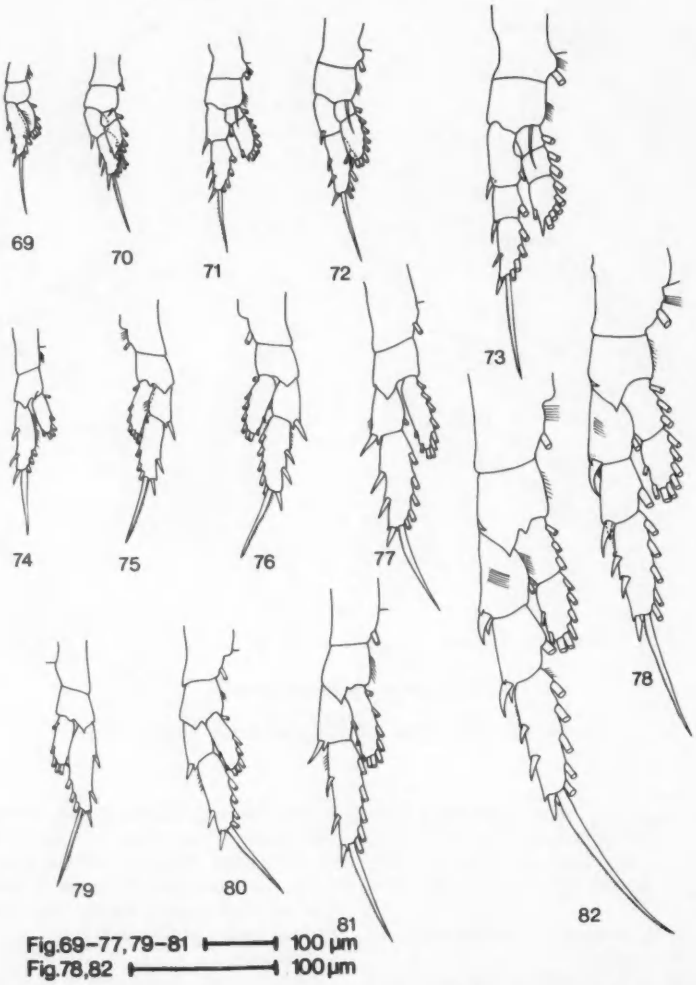


FIG. 69–73. First foot, Copepodid I–V, respectively. FIG. 74–78. Second foot, Copepodid I–V, respectively; FIG. 79–82. Third foot, Copepodid II–V, respectively; FIG. 83–85. Fourth foot, Copepodid III–V, respectively. FIG. 86. Fifth pair of feet, Copepodid IV, female; FIG. 87. Fifth pair of feet, Copepodid IV, male; FIG. 88. Fifth pair of feet, Copepodid V, female; FIG. 89. Fifth pair of feet, Copepodid V, male; FIG. 90. Fifth pair of feet, adult male; FIG. 91. Fifth foot, adult female.

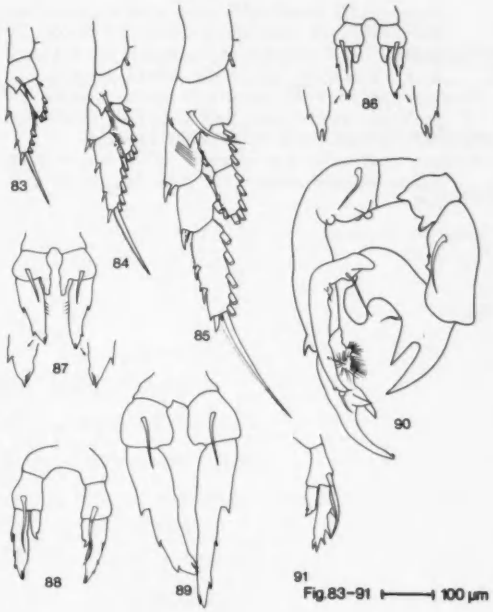
segment. Maxillae, maxillipeds, first and second feet rudimentary. Left caudal spine approximately ⅓ as long as body.

Copepodid I (Fig. 25, 34, 39, 44, 49, 54, 59, 64, 69, 74) — Length 1.10–1.12 mm. Prosome 3, urosome 2-segmented. No lens, no rostrum. First and second, and rudiments of third feet present.

Copepodid II (Fig. 26, 35, 40, 45, 50, 55, 60, 65, 70, 75, 79) — Length 1.24–1.34 mm. Prosome 4, urosome 2-segmented. Lens and rostrum present in this and subsequent stages. First to third, and rudiments of fourth feet present.

Copepodid III (Fig. 27, 36, 41, 46, 51, 56, 61, 66, 71, 76, 80, 83) — Length 1.50–1.74 mm. Prosome 5, urosome 2-segmented. First to fourth feet, and rudiments of fifth feet present.

Copepodid IV (Fig. 28, 37, 42, 47, 52, 57, 62, 67, 72, 77, 81, 84, 86, 87) — Length 1.80–2.14 mm. Prosome 5, urosome 3-segmented. First to



fifth feet present. Sexes distinguishable by size, asymmetry and structure of fifth feet; male fifth feet longer than those of female, with left fifth foot enlarged. Spine-like processes on distal end of fifth feet appressed in male, separated in female.

Copepodid V (Fig. 29, 30, 38, 43, 48, 53, 58, 63, 68, 73, 78, 82, 85, 88) — Length 2.72–3.00 mm (female), 2.36–2.88 mm (male). Female prosome 5, urosome 3-segmented, male prosome 5, urosome 4-segmented. Female antennules symmetrical; male right antennule enlarged. Female fifth feet short and symmetrical, male fifth feet long and asymmetrical; right foot larger than left.

Adult male (Fig. 31, 31A, 89, 90) — Length 2.76–3.20 mm. Size difference and antennule structural differences noted in male specimens collected in field samples. Larger males (high dimorph) with process extending from fused region of right antennule. Smaller males (low dimorph) without process.

Adult female (Fig. 32, 33, 91) — Length 3.00–3.60 mm.

Remarks — Of the seven genera in the family Pontellidae (*Anomalocera*, *Calanopia*, *Epilabidocera*, *Labidocera*, *Pontella*, *Pontellina*, and *Pontellopsis*) complete naupliar and copepodid developmental stages have been described for species of *Labidocera* (Gibson and Grice 1976) and *Epilabidocera* (Johnson 1934). The other genera are mostly oceanic in distribution and apparently not readily culturable. Certain developmental stages, based partly on field collected specimens, were described by Fleminger and Hulsemann (1974) for four species of *Pontellina*, by Björnberg (1972) for *Pontellopsis brevis* (Giesbrecht) and by Johnson (1965) for *P. occidentalis* Esterly. The naupliar stages of *Pontella mediterranea* Claus have been reared in the laboratory by Sazhina (1967).

Nauplii of *P. meadi* are readily distinguishable from nauplii of *Labidocera* by their elongate bodies and larger size. *Pontella meadi* occurs in the Woods Hole area with *Anomolocera pattersoni*, a species for which the developmental stages have not been described. Superficially the nauplii of *P. meadi* resemble those of *P. occidentalis* which, however, lack a mandibular blade.

The nauplii of *P. mediterranea* are very similar to those of *P. meadi* in size, configuration, appendage morphology, and ornamentation. Unlike *P. meadi* the rudiments of the maxillule first appear in nauplius Stage III rather than Stage V, and there are small differences in the caudal ornamentation. Too few copepodid stages of Pontellidae species have been described to permit comparison with *P. meadi*.

In the Woods Hole area *P. meadi* is a summer–fall species. It first appears in neuston samples in July and occurs until late November. It is not known whether the July population is the result of recruitment from offshore waters, or whether it is the result of hatching of local resting eggs that were laid the preceding fall and persisted on the bottom until hatching during the summer.

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PERSPECTIVES

Structured Aquaculture Development with a Canadian Perspective[1]

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Abstract

PRITCHARD, G. I. 1976. Structured aquaculture development with a Canadian perspective. J. Fish. Res. Board Can. 33: 855–870.

Wild fisheries resources in Canada will be increasingly replaced or augmented by aquaculture as they diminish or encounter difficulty in satisfying market demands. Commercial forms of aquaculture still suffer from the lack of innovative policies, well-delineated strategies, and pilot-scale experimentation in operational planning. The aquaculture activities in Canada are reviewed and analyzed in this paper against a model for technological planning and social futures, and against developments elsewhere in food systems. It concludes that substantial research and experimental development effort are still needed, and that the lead time for undertaking these activities is short if risk capital is not to be jeopardized. High priorities are given in support of i) innovative policy development (resource inventories, public anticipations, etc.), ii) strategic forecasting and planning (business strategies, modular enterprise structures, marketing, disease control, a gene pool of aquatic stocks, sea-ranching, aquatic polycultures, introduction of nonindigenous species, waste utilization, etc.), and iii) commercial operations (feed formulation, brood stock selection, water reconditioning, product development, production tactics, pollution control, etc.). There is a shortage of skilled aquaculture practitioners, and a need for more highly trained scientific personnel to address themselves explicitly to aquaculture. In Canada, this industry has already begun to take on an innovative structure that makes it a model for other forms of fisheries development.

Résumé

PRITCHARD, G. I. 1976. Structured aquaculture development with a Canadian perspective. J. Fish. Res. Board Can. 33: 855–870.

L'aquiculture contribuera de plus en plus à remplacer ou à augmenter les ressources halieutiques sauvages au Canada à mesure que ces dernières diminueront ou auront de la difficulté à satisfaire la demande des marchés. L'aquiculture commerciale souffre encore d'un manque de politiques innovatrices, de stratégies bien délimitées et d'expérimentation à échellepilote dans la planification des opérations. Dans cet article, l'auteur passe en revue et analyse les activités aquicoles au Canada en les opposant à un modèle de planification stratégique et à des prévisions sociales, et en les comparant aux développements qui se produisent ailleurs dans les systèmes alimentaires. Il en conclut qu'il y a encore un grand besoin de recherche et de développement expérimental, et qu'il ne reste plus beaucoup de temps pour entreprendre ces activités si l'on ne veut pas que le capital de risque soit compromis. De hautes priorités sont accordées au support i) du développement de politiques innovatrices (inventaires des ressources, expectative du public, etc.), ii) de la prédiction et de la planification stratégique (stratégies administratives, structures d'entreprise modulaires, commercialisation, contrôle des maladies, pool génétique de stocks aquatiques, élevage en mer, polycultures aquatiques, introduction d'espèces non indigènes, utilisation des déchets, etc.) et iii) opérations commerciales (formules alimentaires, sélection des stocks reproducteurs, reconditionnement de l'eau, développement de produits, tactiques de production, contrôle de la pollution, etc.). Il y a une rareté de personnel expérimenté dans la pratique de l'aquiculture, et il faudra qu'un plus grand nombre de scientifiques hautement formés se consacrent explicitement à l'aquiculture. Au Canada, cette industrie a déjà commencé à se doter d'une structure innovatrice qui en fait un modèle pour les autres formes de développement dans le domaine des pêches.

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Introduction

Aquaculture is an emerging bioindustry based upon the culture and husbandry of aquatic plants and animals. It is a significant departure from the traditional "search and capture" fisheries in Canada in that production processes are controlled in whole or in part. This control enables a manipulation of the production processes that would otherwise be impossible, and allows for ownership or tenure as the fish are no longer a common property resource; thus the opportunity to create an innovative new aquaculture industry excites many. Nevertheless, a new spectrum of problems must be faced when cultured plants and animals are reared in large numbers in close proximity one to another. These problems suggest a growing need for new technologies and institutions to assure the technical and economic basis for commercial aquaculture in Canada.

The study reported here was commissioned by the Fisheries Research Board of Canada (FRBC) to provide a background for compiling recommendations on national needs for research and

experimental development related to fisheries. It focuses on the science of aquaculture, its assessment, development, and applicability. It outlines a model for structuring aquaculture development against which priorities and time-frames can be determined for the various enterprise options likely to be followed. It pays attention i) to "the farming approach" to aquaculture that is directed to enterprises that are not overly large, that make better use of natural resources in rural areas, and expand employment opportunities in outlying communities; ii) to "factory" aquaculture embodying intensive and large-scale hatcheries, sea ranches, feedlots, etc., built largely on the sciences of nutrition, genetics, disease control, and environmental engineering; and relates in a limited way to iii) "integrated" aquaculture that opens the whole topic of closed-circuit ecology with the controlled utilization of primary production within suitable water volumes either directly or via a food chain for human consumption. It specifically does not address itself to the ecological problems associated with managing wild fisheries resources, and touches only lightly on those aspects of primary productivity, hatcheries, and food technology that interface with the other FRBC background studies covering biology oceanography, resource enhancement, and resource utilization, respectively.

The study was built around a series of questions being asked with increasing frequency: 1) Is there a place for aquaculture in Canada, and if so, on what basis? 2) What types of enterprises are now being visualized, and what is the scale of these aspirations? 3) What ingredients, including food reservoirs, exist in this country applicable to aquaculture, and how many of these are unique enough to give Canadians a competitive advantage? 4) What is the state of present husbandry practices applicable to aquaculture? 5) How good is the existing knowledge base for aquaculture systems (with special attention to nutrition, genetics, disease control, hormonal manipulation, reproductive physiology, environmental engineering, and the management of food systems) and what should be done to improve this base? 6) What are the institutional, legal, and attitudinal constraints to aquaculture in Canada? 7) What is the case for government involvement in aquaculture research and experimental development in Canada, and what are the priorities? 8) Do the aquaculture sciences have application beyond the food industries? 9) How should "high-science-content" aquaculture be fostered in Canada? 10) How should Canadian activities equate with the international scene?

The study approach consists of a situation analysis that draws heavily from existing reports and activities of recent origin. It involved no special travel or data collection, and gaps that result from this approach are acknowledged.

Background Factors

The overall study was conditioned by a number of factors that should be enunciated at the outset.

1) The place of aquaculture in food systems quickly overshadows the recreational and environmental benefits associated with it, thus an assessment of aquaculture requires some fundamental reexamination of food systems now in use. Food production is very much a chemical process. Basic energy reserves in the universe are almost infinitely beyond the energy delivered to human beings through nutrition. Nevertheless, a great part of the scientific and technical efforts applied to food problems is directed still to technical modification of relatively primitive agriculture and fisheries systems that are usually inefficient and dependent upon high-cost energy inputs. Aquaculture could prove to be the exception. Much benefit must come from assessing the potential that lies in more efficient technical processes for delivering nutritional energy from natural resources to people. At the present time, we can hardly claim to have assessed or inventoried the natural material and scientific knowledge available for making a concerted, long-range international attack on this most basic of human problems, the lack of adequate nutrition (Kenyon 1974).

2) There are four distinguishable and sequential steps in the development of a food system (Jantsch 1972): i) wild growth (e.g. collecting wild fruits, etc., hunting wild terrestrial animals and birds, freshwater fishing, ocean harvesting), ii) farming on a self-sufficiency basis (e.g. agricultural plant production, agricultural animal raising, fish ponds, mariculture), iii) intensive farming (e.g. agricultural plant production with chemical fertilizers, animal feedlots, agricultural plant production under artificial conditions), iv) factory food production (e.g. protein extraction from vegetable or oil seeds, yeasts grown on molasses, fish protein concentrate, industrial production of amino acids).

There are also a number of activities still in the exploratory stage (e.g. carbohydrates from wood, yeasts grown on organic wastes, protein extracted from leaves, freshwater algae culturing, yeasts and bacteria from petroleum). It is significant to note that only 5 of these forms of food production play a major role in the current production of food for human consumption, although 18 are

in current use. In contrast, Jantsch claims 3168 realistic possibilities based from a systemic morphological analysis of the food production function. He concludes that the biggest potentials can be ascribed to the following: i) biological transformation of fossil macromolecular resources (petroleum, natural gas) yielding single cell protein (SCP) with a production potential many times the total protein demand; ii) photosynthetic and, to a higher extent, nonphotosynthetic production of protein-rich algae, yeasts, tissues, etc., under artificial environment conditions (for example, in water tanks); iii) mariculture; iv) transforming the forests of the world chemically (yielding only carbohydrates) or biologically (protein-rich yeasts). Aquaculture practices at the lower trophic levels are tied closely to primary production, whereas those at the secondary or tertiary level become dependent upon outside food sources. Thus, nutrient recycling in aquatic systems must be kept in proper perspective to the aquatic food pyramid described by Ricker (1969), but can be linked to other and newer forms of food production.

3) Animal and plant production systems in agriculture have been under continuous pressures to intensify; defined here as increasing the time rate at which commodities are produced from feed, water or land resources, and labor. The same can be expected to apply to aquaculture. It must come from increasing the number of plants or animals carried per unit of water or labor, and from increasing the productivity of individual organisms. Both involve increased capitalization in production facilities and the capture of technical and scientific advantages. The paradox of this syndrome has been identified by Blaxter (1973) who points out that pressures to intensify food produtcion are greater in those countries of the world that are highly developed industrially than in the developing countries where demand for more and better food is greatest.

4) Limitations to the world marine fish catch are amply illustrated in trends displayed by Gulland (1970) and similar patterns are well-established in freshwater fishes. In contrast, the future potential for an increased production of protein food by aquaculture is very large. While capture fisheries for food fish are not expected to double their present world tonnage, means have already been identified that could prompt a tenfold increase (to 50 million tons annually) in world aquaculture production by the year 2000 (FAO 1973).

5) The driving force behind aquaculture must be first and foremost an economic one. Markets have to be created or found for its products, and the rewards must compensate for the cost of production inputs. Government policies and programs may encourage change, control overproduction, and/or minimize the hardships from temporal situations, but should never encourage forms of aquaculture that have little hope of long-term economic viability. The economics, however, are changing, as the exuberant economics of abundance relative to demand appear to be giving away to the economics of scarcity. Success in coping with such change will be ever more dependent upon scientific and technological capabilities (Handler 1974).

6) Aquaculture is still very much in the development phase, and as such exemplifies the wide range of problems and constraints normally encountered in launching a new technologically-innovative, science-based industry (Pritchard 1973b). This phase is a fast-changing one, requiring broad conceptualization and policy development that is not overly rigid. It could be disastrous for this industry at this stage to be emasculated by pseudopolicies emanating from intuition, or from existing alignments with the management of wild fish stocks.

A Model for Structured Aquaculture Development

The differences between policies, strategies, and operations (tactics) must be brought into sharp focus if meaningful research and development requirements are to evolve. An inordinate confusion exists among these functions. Each must have its forecasting, planning, decision-making, and rational creative action phases, and all three must be addressed if structured and rational aquaculture development is to occur.

A proposed model for structured aquaculture development is presented in Fig. 1. It is adapted from a model described by Jantsch (1972), and provides a framework for the Canadian situation analysis on aquaculture that appears in subsequent sections of this report.

The features of this model particularly applicable to the topic under review are 1) an integration of essential activities, 2) a separation of policies, strategies, and operations, and 3) a dominance of norms, which are derived from values. The latter is equivalent to stating that the scheme recognizes policy making as the guiding function of the entire action process.

The full scheme integrates four vertical columns and three horizontal levels. The vertical columns represent the four activities (forecasting, planning, decision-making and rational creative action), each structured in three horizontal levels (policies,

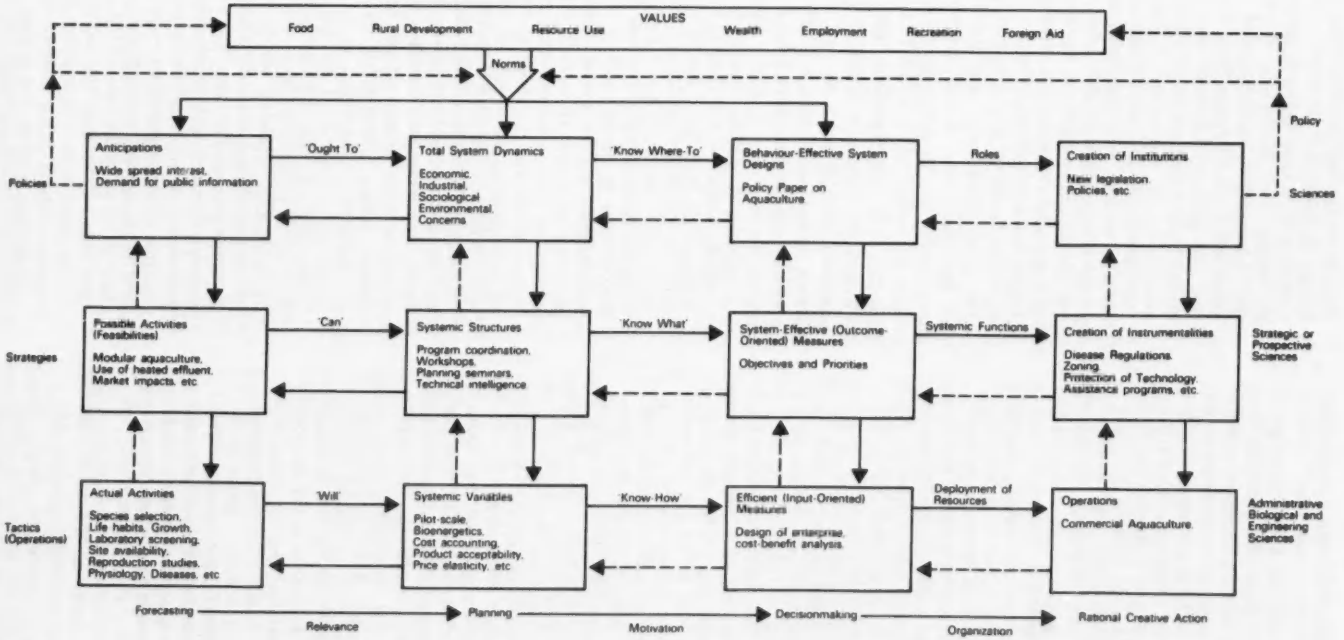

FIG. 1. Structured aquaculture development.

strategies, and operations). Jantsch characterizes them as follows:

> "Forecasting deals with the invention of anticipations, of possible (feasible) activities fitting them, and with the assessment of actual activities. Interaction among the three levels is by continuous feedback processes, but the downward pointing full arrows emphasize the basic normative character of forecasting; for example, a compilation of possible activities cannot determine anticipations, but anticipations, evaluated as 'good' determine the spectrum of possible activities to be considered, within the range of feasibility. Forecasting and planning are tied together particularly closely (since creative planning is based on invention through forecasting), and they shape their policy and strategy constructs together in feedback processes with no really dominating direction. These ties may be called relevance, expressed by 'ought to,' 'can,' and 'will,' that characterize the planning levels.
>
> "Planning deals with system design at the levels of total system dynamics, system analysis to define effective changes in system structures (goals), and changes of variables in given system structures. The feedback interaction among the three levels is of the same nature as with forecasting. Planning provides the information basis in dynamic terms for decision-making. In a normative framework of rational creative action, planning has to provide for links to decision-making which may be called motivation, expressed by the information values of 'know-where-to,' 'know-what,' and 'know-how.' The current dominance of 'know-how' in the motivation of decisions (e.g. for technology) illustrates the grossly distorted balance among the three levels of planning and decision-making. But information on the 'know-what' may also be meaningless, if it is not scrutinized in the light of criteria provided by policies and making it possible to distinguish between 'good' and 'bad' strategies.
>
> "Decision-making deals with the recognition, again in a normative way, of system designs which can be expected to be effective in changing the dynamics of the system in the direction of preferred anticipations and increased dynamic stability, of system-effective measures (whose outcomes do not upset systems in the same way as, for example, the introduction of technology for specific 'single-track' purposes frequently upsets the systems of human living), and of efficient measures to do well what has beed decided to do. The predominance of efficiency and decision-making structures focussing on it, bears testimony to the current distortion in the decision-making process. Decision-making leads to action by means of organization, which focuses at the three levels on the definition of roles, on systemic functions and on the deployment of resources of a material and nonmaterial nature (including the available basis of science and technology, human skills, etc.).
>
> "Rational creative action, finally, deals with the creation of institutions, the creation of their corresponding instrumentalities, and operations within and through these instrumentalities. The growing recognition that institutional change has to precede all other change in social systems, is an expression of frustration by forecasters, planners, and decision-makers alike who see no way to implement their ideas and preferences in the rigidified structures of existing institutions and instrumentalities. It is also an expression of the a-priori necessity of policy design human creative action."

It should be noted that the activities embedded in the process do not constitute disciplines, or even interdisciplines in the conventional sense; thus avoiding the tendency to characterize aquaculture according to traditional disciplines and outlooks. This is compatible with the practical need for planned transdisciplinary approaches enunciated by Regier et al. (1974). More and different aspects come into play when we look at the horizontal layers, defined by Jantsch as follows:

> "At the policy level, we gain a first view of a viable conceptual framework for the policy sciences. We may say, the policy sciences deal with the horizontal unfolding, as general instances of human activity, of forecasting, planning, decision-making and action at the policy level. What is meant thereby is more than strengthening the horizontal coupling between the four activities. It is their conformity in conception and realization, even their identification, which must be aimed at. The invention of anticipations, the design of social systems with preferred dynamic characteristics, the definition of roles for and the creation of institutions, and the feedback interactions between them, have to be seen as one single task. The emphasis is on design: system design, policy design, design of institutions; in other words, on intellectual innovation.
>
> "At the strategic level, we find a conceptual framework for what we may tentatively, and for lack of an accepted term, call the strategic or prospective sciences, which have not yet become recognized as such. However, the task of dealing integrally with the conception of possible activities, their implications for system structures and system-effectiveness, and the creation of instrumentalities — in other words, the human action model of structuring rationality at the strategic level — has already come into better focus than the corresponding task at the policy level. The emphasis is on analysis: system analysis, policy analysis, need analysis, institutional analysis, and market analysis.
>
> "At the operational level, the corresponding task of integrating probablistic forecasting related to actual activities, the changing of system

> variables in given system structures and efficient ('economic') operations is pursued in a most effective way by the management and administrative sciences (biological and engineering sciences related to aquaculture are also accommodated here). The emphasis is on expansion: expansion of actual activities, of system variables, of resources, markets and operations in general."

The correct and logical order of the process of rational action according to Fig. 1 is to proceed from the left to the right, and from the top down. In practice, all too frequently people prefer to proceed in the wrong direction, especially in the direction from the bottom up which results in rigidification of existing system structures, and a short-circuit of the normative thinking.

Values vs Norms

The answer to whether there is a place for aquaculture in Canada, can only be found by examining the discrepancies between "Values" and "Norms." These determine human activity, and dictate a consideration of social as well as economic factors, although the pace of development is determined by success in defining competitive bases for enterprises. This analysis also provides criteria for research planning and evaluation.

Aquaculture, as it is being conceived in Canada, contributes in varying degrees to several values which have existing norms that are deficient, summarized below:

Food

Canadians have come to expect and appreciate inexpensive, high quality, and variety foodstuffs, but increasingly have become disenchanted with what is being offered. Aquaculture promises specialized products that could satisfy consumer specifications with variety and quality unobtainable through traditional fisheries. Even when traditional fisheries products encounter cyclical market difficulties, a demand for products most likely to be produced by aquaculture go unsatisfied. The origin of nutritional problems is in the limitations of food production capacities and techniques (Smith 1970), thus research and production planning must direct itself effectively toward activities that contribute to providing nutrition for those who must eat economically if they are to eat well.

Rural Development

Growth of industrialization and urbanization in Canada was to a large extent at the expense of rural areas that suffered population drains, constraints on investment, and renewable resource neglect. These trends are reversing, and the assets of rural Canada are being recognized both properly and more widely as governments seek distribution of social and economic benefits to rural areas. New opportunities and instruments for rural development are being actively sought. Aquaculture and associated services loom high on development among such opportunities, and there is active search within business circles for rural investments that could be financially self-sustaining.

Resource Use

Greater attention is being paid to seeking rational use and maximal benefits from natural resources without destroying their productive capacity. In the past, norms for use frequently tended to be exploitative, with few rewards being returned to strengthen the natural resources. Aquaculture is a form of renewable resource production that is compatible with good environmental guidelines, although uncontrolled development could pose threats to natural populations through disease introductions, genetic mixing, and the build-up of organic wastes.

Wealth

Food exports generate a substantial proportion of Canada's foreign exchange, thus creating new wealth which Canadians value and wish to maintain. The present outlook for fish markets is uncertain, but the expectation is for export markets to become more specialized and demand rigorous specifications, making markets increasingly difficult to penetrate and hold. Aquaculture could be used to reduce dependence upon single sources of supply and to build-in flexibility for using good ingredients in ways current market economics dictate; thus enabling the retention of markets for Canadians that might otherwise be lost.

Employment

Canadians continue to seek wider opportunities for meaningful and productive employment. Employment options are particularly limited in remote areas where aquaculture might be feasible.

Recreation

Canadians continue to seek outdoor recreation, but recreational fishing is often unavailable in the proximity of population centers and established resorts. Correction of this situation is dependent upon specialized forms of aquaculture.

Aid to Developing Countries

Canada's programs of aid to developing countries have a sizeable aquaculture component, but the demand from developing countries for Canadian expertise related to the specialized sciences cannot be met.

Anticipations — Total System Dynamics

There is a great variety of types of aquaculture enterprises now being visualized in Canada, and some have led to large investments. Others are less costly. Often the normal pattern of aquacultural aspirations is for them to be highly imaginative, short of facts, and underfinanced. On the other hand, private investors who seriously wish to develop Canadian aquaculture have been generally frustrated by a lack of clarification of their legal status, by a lack of biotechnical data from pilot-scale operations upon which reliable cost-return projections can be built, by risks associated with inadequate disease control, and by the unavailability of suitable fish for foundation stocks. They find it difficult to identify centers within governments from which they can obtain information, and that much of the information available is inadequate or irrelevant. Press reports tend to be overenthusiastic and misleading. At the same time there are jurisdictional areas within governments reflecting various economic, industrial, social, and environmental concerns that identify with aquaculture in some way. Moreover, the aquaculture client is frequently unknown to even the fisheries sections as but a small percentage of the aquaculture anticipations are generated by people within the traditional fishing business. The resulting action usually takes one of three routes: i) the anticipated plan is aborted based on information obtained in a great number of cases. This presupposes that the information demands can be met; ii) requests are sent into the political arena seeking blanket endorsement and support, but such proposals are frequently ill-defined and premature; iii) a plan evolves for examining the feasibility of the enterprise proposed; this being the normal progression for those anticipations that are most promising.

Possible Actions — Systemic Structures

Feasibility studies on aquaculture must address themselves to the question as to what ingredients, including food reservoirs, exist that are applicable to aquaculture, and how many of these are unique enough to give a competitive advantage in the market place.

There are few formats for planning a commercial aquaculture feasibility study, but some exist (Mayo 1974; Beamish et al. 1975). Regardless of the format, the first requirement in an aquaculture endeavor is to identify a site. Feasibilities are meaningful only if they are site oriented. This is particularly true in the self-sufficiency and intensive-farming forms of aquaculture that rely to a much greater degree on the natural ingredients than in factory food production. All, however, must consider factors such as water quality and quantity, conflicting uses for the site including contamination from other activities, and cost. In general, Canada has large areas of indented coastline and abundant inland waters that are relatively clean and suitable for aquaculture. Few of these, however, have been inventoried with a view to selecting possible sites for aquaculture, and to preventing their deterioration so that potential bioindustries will be able to operate. Also, suitable quantities of ground water can be of critical importance to freshwater aquaculture if expensive recycling and water restoration procedures are to be avoided.

The second requirement is to determine if an enterprise encompasses the managerial, scientific, and technical skills required for its operation. The lack of clear-cut business plans and of skills to implement an operation is a likely cause of failure. This is particularly critical on the Canadian scene where there is but a limited history in the husbandry of aquatic species, and operational guidelines must frequently be extrapolated from the agricultural sector where nutrition, genetics, and disease control is further developed, or from foreign technology.

If the first two requirements are met, one would be expected to proceed toward the design of a production system, the component parts of which are identified in Fig. 2. Input ingredients vary greatly with the nature of the enterprise, so emphases shift accordingly. In a self-sufficiency type of enterprise (e.g. oyster, mussels, etc.), strategies are built around the capture of the sustaining food sources, whereas in intensive forms of aquaculture (e.g. trout and salmon) feed supply and cost problems often dominate. Selection of animal species must be made on availability, ease of husbandry including the ability to reproduce in confinement, resistance to disease and predation, food conversion capacity, and product value (Jones 1972). Machinery and motor power options range all the way from stationary floating suspensions to fully controlled environmental systems with automated material handling, the latter being the forecasted trend in factory-food production. Power costs can be pro-

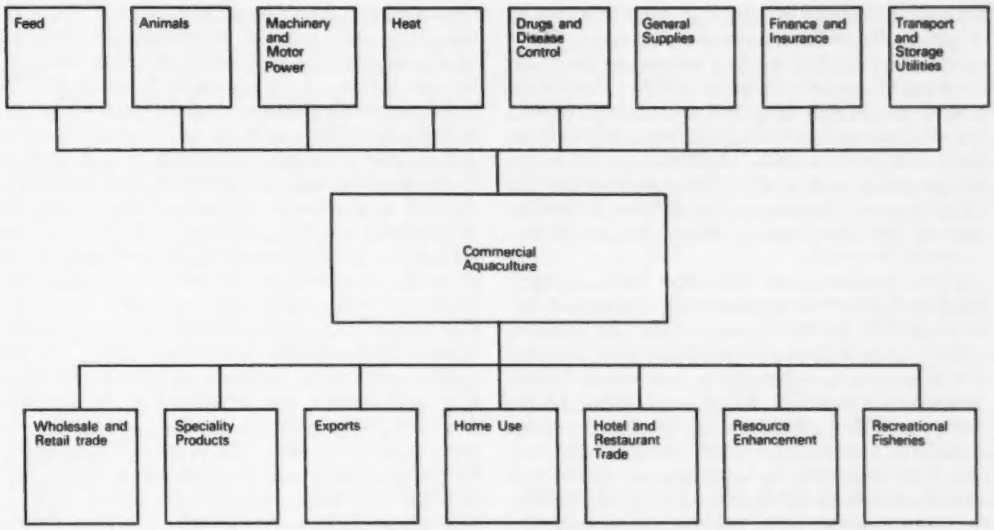

FIG. 2. The aquaculture production system.

hibitive, so the availability of low-cost heat from other industries has spurred considerable interest in developing aquaculture using waste heat effluents, as from nuclear power generating facilities. A plan for prevention and control of infectious diseases is a most vital ingredient of aquaculture ventures to minimize risks. General supplies, finance and insurance, and transportation, storage etc., are all cost factors that must be accommodated.

Market studies to assess the various marketing options are also required. Specialty markets tend to demand continuity of supply with price stability, thus timing of harvest and distribution capabilities can become a key factor in production tactics.

Looking at the broader picture, numerous strategic studies applicable to new aquaculture ventures have been conducted with government support and are a matter of public record. Others, however, were made at private expense and became privileged reports. Together, they cover a wide spectrum of activities and areas within Canada, only a brief summary of which can be provided here.

Oyster rearing, an industry of long standing on both coasts (Metcof 1961; Quayle 1969, 1971) has been recently rejuvenated with the injection of new techniques (Environment Canada 1974). Aquaculture ventures in Lake Bras d'Or, for example, have been heavily oriented to oyster farming although mussels and trout are also being grown experimentally. Marine algae were subject to much study in Eastern Canada (Neish 1972; Federal/Provincial Atlantic Fisheries Committee 1971; French 1971) and commercial ventures are becoming feasible. A major study on use of waste heat effluents was conducted for the Lorneville, New Brunswick, site (MacLaren Atlantic et al. 1974), other sites have or are being examined (Shields and Veinot 1969) (a study is presently underway on Point Lepreau). Experimental studies on mussels (Jamieson et al. 1975), eels, scallops (Clugston 1974), lobsters (Wilder 1972), and salmonids in salt water (Macdonald 1974; Saunders et al. 1974; Sutterlin et al. 1975) have been conducted in the Atlantic area, but only one commercial trout hatchery operates there at the present time. This contrasts with Ontario and Quebec where several hundred private trout hatcheries operate, all on a small scale. Many of these operations are looking toward grow-out operations as a form of expansion. Trout farming differs again in the Prairie lake region where natural populations of *Gammarus* serve as a prime foodstuff, attracting considerable attention and a growing number of enterprises (Lawler et al. 1974). Trout were also experimentally reared in cages in Precambrian lakes (Whitaker and Martin 1974), and use of waste heat effluent for freshwater aquaculture is being investigated in Ontario. British Columbia also has established trout farms that operate in the private sector, and a growing interest in sea pens for salmon is de-

veloping (Kennedy et al. 1975; MacCrimmon et al. 1974). Sable fish culture was attempted (Kennedy 1974; 1972), as has culture molluscs in addition to oysters (Bourne 1972). Feasibility studies on the fish feed industry (Bissell 1974), and numerous marketing analyses have been made. Tests have been underway in both the private sector and at the Nutrition Department, University of Guelph, on possibilities for using various feed ingredients produced in Canada for salmonid feedstuffs.

The Canadian scene has also been strongly influenced by the strategic studies and industrial developments in the United States on various culture systems now considered ready for exploitation (e.g. Gaucher 1971; Gates et al. 1974; Mardela Corp. 1972; Klontz and King 1975; Novotny 1975; Ryther et al. 1972a, b). As early as mid-1973, when aquaculture potentials in Canada were reviewed at a seminar in Winnipeg, species considered ready for pursuing vigorously on a commercial scale included chinook, coho and sockeye salmon, rainbow trout, and Atlantic and Pacific oysters. Meriting full-scale production subject to verification of product quality and of special market demand were species including pink and chum salmon, Atlantic salmon, sablefish, eels, and freshwater crayfish. Species that should be cultured on a small scale until further technical and/or market data were obtained included flatfishes, walleye, whitefish, clams, quahaugs, mussels, scallops, lobsters, and seaweeds. If a breakthrough were to be obtained in the culture of lobsters, pay-off could be extremely high.

Upon review of activities concerning aquaculture feasibilities, one is impressed by the multiplicity of expertise being addressed to the subject. There seems to be no shortage of people who are willing to undertake aquaculture studies, and are anxious to develop their expertise in this area. For example, a recent contract call in Ontario solicited 11 proposals, with representation from all regions of the country, each proposal being backed by a considerable array of expertise. Also, a conference on aquaculture in the Bras d'Or sponsored by the Atlantic Provinces Inter-University Committee on the Sciences attracted about 250 registrants, suggesting that there is a large amount of scientific and technical expertise now underutilized that is looking to aquaculture to find new and meaningful challenges.

At the same time, one becomes distressed by the lack of institutions that consider aquaculture in an explicit way. In the past, this has all too frequently resulted in the exclusion of potential users, an improper mix of disciplines, results that were nonadditive, and a distorted division of responsibilities. Steps have been taken to alleviate this lack of systemic structures. Workshops were conducted for staff within the Fisheries and Marine Service, a Government/Industry Policy Development Seminar on Aquaculture was conducted (MacCrimmon et al. 1974), and the subject is under ongoing discussion with Federal/Provincial meetings. The Service also moved to establish a coordination office in Ottawa, but its effectiveness has been curtailed by the slowness with which parallel regional structures were put in place. It has also been difficult dealing with the existing industry which lacks a collective voice. This general problem, however, is well-recognized in the fisheries sector. Rothschild (1973) noted it when addressing questions of strategy in fisheries management and development. He emphasized the importance of explicit institutions in creating a favorable environment for strategic decision-making, and identified the solution. Of particular relevance, in his view, is the applied research institution to develop strategies. Most would concur.

Actual Activities — Systemic Variables

Operations, or tactics, have been pointed out by Mackenzie (1974) to be a specialist's activity. Bardach et al. (1972) identified a four-stage sequence of this specialized activity for aquaculture that ranges through 1) preliminary screening for the selection of new species under subsistence cultivation only, 2) scientific screening (laboratory studies), 3) extension of laboratory research to pilot-scale, and 4) commercial trials. These stages coincide closely with the operational forecasting and planning phases of the model in Fig. 1.

Scientific activities in Canada have been heavily concentrated in the screening stages and on the background biology. In contrast, the number and breadth of pilot-scale and commercial trials undertaken within the private sector lacked a well-developed scientific input, both in the form of information and of experienced people. There have been serious gaps in the understanding of nutrition, applied breeding, animal behavior, and disease control technology. Copying government hatchery operations was also found to be inadequate for the private sector as here, too, scientific content was frequently weak, with facilities being planned and put into operation with little foresight as to genetic capacity of broodstocks, feed supplies, and the disease status of the watersheds on which they operated.

This operational pattern is somewhat ironic when considered relative to happenings in the

scientific community where aquaculture, still somewhat in its infancy, has attracted and continues to attract some of the best scientific minds in the country. A high percentage of senior scientists who work on fish within the Fisheries and Marine Service have aligned themselves with the Aquaculture Program, bringing to it a scientific credibility as high as enjoyed by any other fisheries program in Canada. This cannot be attributed to stimulation by funding; it is probably the result of the lack of intellectual challenge that tends to creep into programs of longer standing. One might reasonably have expected funding constraints to dampen exploratory research related to aquaculture. It has been increasingly difficult to obtain either private or public financing for research and experimental development that cannot be recouped on the market place within a reasonable period of time. However, such periods of austerity have tended to force scientists to work on laboratory-scale studies that are less costly, but also less relevant, than those that should be conducted on a pilot-scale. In the meantime, Canada has built up a unique body of highly expert professionals who feel frustrated at times because they are far from the policy making centers, and because they have lacked avenues for following the identified opportunities through the step-by-step experimental process that would lead to commercial operations.

To correct this situation, the Fisheries and Marine Service of Environment Canada proposes to embark on an intensified 5-year program to strengthen the technical and economic basis of commercial aquaculture in Canada (Lucas 1975). The program will include the testing and economic evaluation of pilot-scale feeding and culture systems, the establishment of a fish health protection plan, and selection and provision of foundation fish stocks that have been performance tested.

Behavior-Effective Systems Designs — Creation of Institutions

The Fisheries and Marine Service, Environment Canada, with Government approval, is also proceeding with the formulation of an all encompassing national policy for commercial aquaculture (Lucas 1975). A first step is the preparation of a policy paper that will review and make recommendations for controlling commercial production, licensing, site tenure, water rights, confiscation and compensation for diseased stocks, marketing, and the possible application of existing government programs designed to assist farmers and fishermen. The paper must be prepared in consultation with Agriculture Canada and other departments of federal and provincial governments. Its purpose is to assist Cabinet in defining the nature of future federal involvement in commercial aquaculture development. As such, it will have to include a discussion on the appropriate degree of provincial participation in the financing and administration of future policy initiatives, an acknowledgment of the institutional constraints in fisheries management discussed by Thompson (1974).

This action represents a significant turn of events which could lead to much greater private initiative and involvement. Traditionally, federal fisheries authorities have been indifferent to what is termed commercial aquaculture, and operated almost wholly to manage the common property fish resources. Similar outlooks often prevailed in the provincial sector. Thus, government programs that evolved were oriented to the conservation and exploitation of natural fish resources with little attention to production in the private sector. In contrast, agriculture was built on well-established systems of ownership or tenure of production sites. Commercial aquaculture falls between these sectors with the result that people are confronted with uncertainty when mounting enterprises. The legal status of aquaculture is not specifically addressed in federal legislation. In practice, the conflict between public attitudes and values concerning recreation, wildlife, resource management, and commercial aquaculture was too often unjustifiably reflected in negative attitudes by governments toward the latter.

Even before this national policy review was begun Environment Canada was examining the policy options open for aquaculture in Canada, and the implications associated with these policies. The policy that evolved between 1972–74 can be summarized as follows:

The prime role identified for the Fisheries and Marine Service was in research and the provision of scientific and technical information designed to reduce risks encountered by aquaculture enterprises. Such projects were to be strengthened as funds became available, with project planning consolidated within regions due to the site specific nature of most aquaculture projects.

Uninhibited shipment of live fish lacking disease certification was recognized as presenting undue hazards to both confined and natural fish stocks. Governmental action was needed to implement effective prevention and control procedures for communicable fish diseases, and steps were initiated to establish a scientific basis for an effective fish health program.

Speculative information and excessive promo-

tion of aquaculture was to be avoided pending establishment of an adequate scientific–technical base.

The supply of risk capital available to entrepreneurs wishing to launch or expand aquaculture was to be reviewed but direct financial assistance was to be avoided with the exception of a modest federal–provincial shared-cost program already underway.

The Freshwater Fish Marketing Corporation agreed to follow a liberal policy of licensing the sale of aquaculture products so that investors would be able to have full benefits of direct access to market place.

Where applicable, provincial water rights and other relevant licenses were to be considered a prerequisite to federal encouragement.

Undue regulation beyond those steps necesssary to ensure that harmful substances were not introduced in the fishing grounds was to be avoided. Priority was to be given to developing a strengthened fish health program with a private enterprise orientation, and the concerns for genetic swapping and for the destruction of prime aquaculture sites were identified.

Canadians should avail themselves of opportunities to seek out new technology from other countries.

A fish food manufacturing industry in Canada was to be encouraged.

Fish stocks from federal sources were to be sold to private enterprises if supplies permitted, subsequently interpreted as eggs from enhanced stocks.

The Foreign Investment Review Act would apply to aquaculture ventures unless direct control of fishing vessels was involved or there was a permanent reliance on the public property fish resources.

U.S. Aquaculture Development

It was soon apparent that these policies were incomplete and inadequate in a rapidly changing scene, but it would be premature to forecast the outcome of the aquaculture policy review and to delineate the institutions that may have to be established to implement new policies. It is of interest, however, to look at happenings in the United States and at the National Aquaculture Development Act of 1975 that came before the 94th Congress. If enacted this Act could influence the competitive position of Canada. Its purpose is to provide for a U.S. program for aquaculture development to increase sources of marine protein for the consumer, to increase the availability and quality level of consumer fishery products, to develop new resources, to improve or maintain recreational fisheries, to initiate new business, industry, and employment, and to provide other national benefits. Activities that would be enabled by this Act include: i) construction, operation, and maintenance of hatcheries or similar facilities and undertaking of natural habitat improvement activities; ii) assistance to public and private organizations and individuals interested or engaged in aquaculture through advisory and other services; iii) coordination of aquaculture activities with efforts to enhance wild commercial and recreational fish and shellfish stocks; iv) development of therapeutic substances for control of fish and shellfish diseases; v) consultation and cooperation with Federal, State, and local governments, regional commissions, institutions of higher learning, private industry, and other public and private organizations for the development of new aquaculture technology; vi) research and experimentation with utilization of waste products (including thermal effluents) for aquaculture; vii) development of improved, new, and economical sources of nutrition for fish and shellfish growing; viii) development of centralized information retrieval and dissemination systems; ix) investigation of legal and regulatory constraints inhibiting the development of aquaculture; x) identification of economic feasibility factors for aquaculture, assessment of economic feasibility for particular species, and development of scientific and technical handbooks and operating manuals for aquaculture; xi) inventory of public and private aquaculture in the United States to include statistics of acreages, gallons of water flow, production in pounds and numbers, techniques used, and unresolved problems which private enterprise cannot solve by itself; and xii) performance of basic and applied research to establish a sound information base for the development of aquaculture.

Many of the powers this Act proposes are already enabled in Canada under the Fisheries Act, and development spending in Canada could be undertaken without passage of further legislation. The enactment of a comprehensive Aquaculture Development Act, however, still remains as one potential solution to many aquaculture development problems in Canada.

System-Effective (Outcome-Oriented) Measures — Creation of Instrumentalities

Aquaculture, as an option in fisheries development, is now recognized within strategic decision-making centers as never before, and its acceptance ensures its consideration relative to other activities in a continuing way. Federal and provincial fisheries agencies identify with it in their objectives and spending priorities to some degree.

Numerous firms have invested in exploring aquaculture opportunities, others wait until they consider that the timing is right.

Assessing the amount of commitment to aquaculture is difficult, as accounting procedures seldom fully separate it from other activities. Fisheries and Marine Service has established programs costing approximately $2,000,000 annually that relate to aquaculture not exclusively, but to some degree. Continuation of these programs throughout the next 5 years is foreseen, and plans formulated to supplement them includes an intensified 5-year program estimated to cost slightly under $6,000,000 in total. Expenditures have also been made on aquaculture by other federal agencies including the National Research Council, the Department of Regional Economic Expansion, and the Department of Indian and Northern Affairs. Provincial expenditures are even more difficult to measure, as plans are only now being expressed.

The largest single component within the Fisheries and Marine expenditures relates to fish health. In total, it is almost as large as the other aquaculture related activities combined, and is accounted for in part by the belated recognition of this serious problem. The case for a strong fish health program is well documented (Bell and Margolis 1972; National Academy of Sciences 1973; Dill 1972). Steps have already been taken to strengthen the scientific basis for disease control (Gillespie et al. 1974), and new regulations are pending. These call for certification of production facilities rather than shipment, extension to 12 diseases from 2, and application to interprovincial shipments for the first time.

Other instrumentalities are less advanced, and must await the outcome of the policy review.

Efficient (Input-Oriented) Measures — Operations

Ideally, any contemplated aquaculture venture should receive a careful analysis of expected benefits and costs. This is true whether the decision lies with a private investor or a public agency, although criteria for evaluation of benefits may differ somewhat between the two. Gates (1971) pointed out that criteria fall into two distinct categories — biologic and economic. The biologic criteria include environmental adaptability and suitability for intensive culture, the latter including a consideration of the present state of cultural knowledge. The economic criteria include market volume and cost–price considerations, interregional competition and natural supplies.

In practice, the knowledge base for even superficial cost–benefit analysis is frequently lacking, and the element of unknown risk is tied directly to this knowledge gap. Ventures that have been started tend to stay small until this risk factor is reduced, and in most cases this seems highly appropriate. On the other hand, undue emphasis on efficiency can discourage innovative ventures, and cause opportunities to be forfeited.

Although the small-scale pattern of Canadian aquaculture ventures described by Pritchard (1973a) still prevails, some changes are on the horizon. Marine algae, trout, and oyster enterprises now have sufficient basis to warrant expansion, although scaling-up problems are still likely to be encountered. Credit is generally scarce, but not surprisingly so in light of the shortage of good pilot-scale data and guidelines within which enterprises will have to operate. The outlook for these gaps being filled in the near future is good, and there is, at the moment, a strong incentive for people to enter the aquaculture business to establish a competitive advantage over those to follow.

Aquaculture in Nonfood Systems

There is an extremely close relationship among the resource-based industries (Weir 1971). Thus, it would be inappropriate to consider a situation analysis of aquaculture within the food system to its complete exclusion elsewhere. It is the intention of this report to identify only the broad perspective that should be maintained.

Recreational fishing now attracts in excess of 4 million participants annually, and surveys show a consistent growth trend (Environment Canada 1975). There is an increasing tendency for the fish stocks on which this industry is based to be sustained by some form of culture. Likewise, the commercial fisheries for Pacific salmon are becoming more and more dependent upon government hatchery operations. Some such hatchery operations may, in the future, find their way into the private sector, provided that a capability grows there which could produce fish for stocking public waters to rigorous specifications.

The hobby fish industry in Canada is largely dependent on imports; very few exotics are cultured for sale. Several major public aquariums enjoy popular support and continue to add to their collections. Culture of fish for live bait used in sportfishing has established itself as a viable industry in areas where its use is permitted. There is also a growing demand for live specimens for research and educational uses. These demands are met largely from natural stocks, although holding and off-season distribution has become established in Canada.

The International Interface

Aquaculture in Canada also must recognize the international implications, as much is happening in both the developed and developing countries. Canada encourages other countries to seek options for fisheries development that might lessen the pressure on fisheries off Canada's coasts. Also, a new regime of marine resource utilization that could emerge as a result of the Law of the Sea Conference clearly points to the greater need for developing fisheries resources within national boundaries. Thus, it came as no surprise that one recommendation to come out of the Technical Conference on Fisheries Management and Development of the Food and Agriculture Organization of the United Nations held at Vancouver in 1973 was for the development of aquaculture techniques, and for strengthening coordinated regional research activities related thereto.

Since 1973 FAO, in cooperation with the United Nations Development Plan (UNDP), has developed an Aquaculture Development and Coordination Program, global in scope. Research programs have been launched, receiving support from several international agencies and Canadian funds through the Canadian International Development Agency (CIDA). The Technical Conference on Aquaculture to be held in Japan in 1976 is an extension of these activities. The International Development Research Center (IDRC) also supports aquaculture research. Canadian expertise is now in demand for these programs.

Canadians have also moved to establish closer ties with aquaculture in the European area, through membership in ICES and its working party on mariculture, by sending observers to EIFAC meetings, and through Science/Technology exchange agreements.

There are also specialty areas which relate to aquaculture, e.g. a consultation was held in 1974 on an International Convention for the Control of the Spread of Major Communicable Fish Diseases, and action on the Draft Convention is still pending. Closer to home, the Great Lakes Fishery Commission developed a policy on disease control which has considerable significance to Canada. In general, benefits are to be gained by building on foreign technology where applicable, but also by not becoming too dependent upon it.

Conclusions

Aquaculture in Canada commands much interest and expectations run high. It is still, however, very much a developing industry. Activities to date, when examined against the model and related background factors, present few surprises. Developments are following a predictable pattern of human activities, and these are likely to be continued because they result from basic values and demands that go unsatisfied. These values relate to food demands (quality as well as quantity), rural development, resource use, generation of exports, employment opportunities, recreation, and aid to developing countries.

This paper considers primarily the food concerns, and it notes that the sequential steps that can be identified in the development of food systems (wild growth, farming on a self-sufficiency basis, intensive farming, and factory food production) are being followed in aquaculture development. Intensification, and more than one form of food production at a time, are trends to be expected when wild resources diminish or encounter difficulty in satisfying market demands.

Much effort on aquaculture has already been made in Canada, and the momentum gained should not be lost. Structured aquaculture development requires an integrated approach if successful operations are to occur. For example, the best of operational planning can be frustrated if instrumentalities of governments are not put in place simultaneously, or vice versa. Thus, research requirments are identified in the three functional areas relating to policies, strategies, and operations, respectively, and priorities should reflect all three. Much of this research, particularly that in support of strategies, would have application well beyond commercial aquaculture.

The single most important requirement for aquaculture is innovative policy development. Research is required to keep it innovative, and to ensure that the instruments of this policy are sound and responsive to change. Policy development involves the design of dynamic systems that will work. Such design requires information inputs based largely upon resources inventories (e.g. zones suited for aquaculture, water resources, foodstuffs, disease status of watersheds, genetic capacity of native stocks, conflicting uses for prime aquaculture sites, market potentials, availability of risk capital, legal constraints, etc.), public anticipations, and overall government policies of the day. Policy development also requires evaluation of the existing information base so that changes will encompass new disclosures, and a capability to assess the system as a whole so that the impact of changes can be forecasted.

The second area involves strategic forecasting and planning, together with the creation of instrumentalities of control or strategic change. This is an important role of governments, and should be oriented to the public good. Strategic planning is first and foremost an applied research activity, a

fact that is all too frequently overlooked even by planners themselves. Such applied research requires institutions that address the strategic problems that infringe on aquaculture in an explicit way; these institutions should be staffed with persons schooled in the systematic collection, analysis, and evaluation of scientific evidence and who have learned to work in a multidisciplinary milieu. Projects of strategic significance that relate to aquaculture and warrant priority in spending plans in Canada include: studies on integration of aquaculture with existing industries, modular enterprise structures for minimizing risks, alternative marketing strategies, disease eradication and protection against communicable disease organisms now foreign to this country, a gene pool for aquatic stocks, sea-ranching, aquatic polycultures, introduction of nonindigenous species, and the utilization of wastes, including domestic sewage, in freshwater aquaculture. Instrumentalities requiring scientific and other services include disease control regulations, licensing and zoning regulations, protection of property (including intellectual property, unique strains or breeds of plants and animals, etc.), regulations against production in excess of markets, and technical assistance or extension programs.

Research in support of operations is preferably done in close conjunction with industrial or pilot-scale operations if not incorporated right into the industrial sector. Such research tends to be more short-term than that in support of strategies, and on a narrower base. If supported financially by governments, care should be taken to avoid unfair competition among private firms. An alternative structure is the creation of an industrial association to undertake and manage research projects in support of aquaculture operations. The highest priority research needs in this area are the formulation of least-cost, high performance fish feeds; selection of disease resistant and high performance brood stocks; the development of water reconditioning and recycling systems; product development; least-cost production tactics; and pollution abatement. Similar problems are encountered by aquaculture operations in many parts of the world, and an industrial capability for researching these problems could also be marketable.

Many manpower resources for undertaking initiatives in the above areas are available in Canada, although there is a need for a greater number of the highly trained scientific personnel to address themselves explicitly to either strategic or industrial problems. There is a shortage of skilled aquaculture practitioners, so greater vocational training is needed.

The level of investment in research and experimental development now undertaken or contemplated is well justified, and likely to grow as enterprises become more competitive for investment capital. Risks to the wide variety of approaches can only be reduced by substantial research inputs. With the inevitable growth of this industry, and the numerous benefits that it offers, governments could readily justify greater expenditures on research and experimental development related to commercial aquaculture. Delay in establishing essential policies and a sound scientific basis for this industry will jeopardize venture capital, and this should be avoided. An excellent start has been made so that aquaculture has already begun to take on an innovative structure making it a model for other forms of fisheries development, here and abroad.

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BOOK REVIEWS / COMPTES RENDUS

The Biology of the Indian Ocean. *Bernt Zeitzschel [ed.] Springer-Verlag, Berlin, Heidelberg, New York. 1973. 549 p. $50.50.*

This collection of 44 papers was presented at a symposium held at the University of Kiel in 1971. The aim of the symposium was "to summarize present knowledge of the biology of the Indian Ocean." The results presented arose largely from the International Indian Ocean Expedition (IIOE) which took place during 1959–65. The papers are grouped under six headings: 1) Environment (6 papers); 2) Primary production and standing stock of phytoplankton and bacteria (8 papers); 3) Standing stock and distribution of phytoplankton, zooplankton, and particulate matter in selected areas (4 papers); 4) Standing stock and distribution of zooplankton (19 papers); 5) Distribution and ecology of fish larvae, fish, and benthic organisms (5 papers); and 6) Transfer of organic matter in different trophic levels (2 papers).

The papers range in substance from comprehensive papers dealing with the entire region (of which there are far too few), such as Wyrtki's paper on "Physical Oceanography of the Indian Ocean," through papers concerned with more restricted geographic areas and subjects, to those that are only brief notes.

In view of the work of Ramage and his associates, the absence of a major contribution in meteorology is surprising. Papers dealing with zooplankton make up about one third of the volume. Papers concerned directly with fishes and fisheries are disappointingly few, but perhaps not surprisingly so in the context of the IIOE. Suda's paper on "Tuna fisheries and their resources in the Indian Ocean" (which is not based on IIOE data) is actually the sole fishery paper.

Dietrich, in his opening paper on "The unique situation in the environment of the Indian Ocean," says of the IIOE: "Now, six years after the expedition, in which twenty nations with forty research vessels participated, we can say it was successful. It is true that not all expectations were fulfilled, mainly because the observations in large areas were not very extensive. Perhaps there would be no criticisms if the expedition had followed the plan made by Wüst (1959) which is based on a schematic grid system of sections with repeated observations in different seasons. Such a plan is ideal but unrealistic. The IIOE had to be worked out by scientists from many countries who took part voluntarily and were interested in a variety of scientific programs. Some regions are extremely rich in problems, e.g. the monsoon effect which can be studied only in certain areas of the Indian Ocean, and some regions lack specific problems, like the southern Westerlies in the Circumpolar Current."

It seems to me (with the obvious benefit of hindsight) that the critical point is not the difference between the rather loose cooperative approach which is actually obtained, on the one hand, and the Wüst-type grid system of observations on the other. Rather, the question is whether the same results, plus needed results in fisheries, could have been obtained more efficiently and without the loss of freedom of inquiry on the part of the individual investigators. I believe the answer to be in the affirmative, but realize it is unlikely that the necessary resources would have been at the disposal of a single institution or country and perhaps unrealistic to expect that sufficient institutional or national sovereignty would have been given up to permit a truly cooperative IIOE. Among the expectations not fulfilled were those relating to fisheries, as has been pointed out by Panikkar (1966) and Marr (1972).

This is an interesting volume and it does bring together a lot of information. However, it cannot be considered to be a complete summary, and certainly is not a synthesis. Perhaps the volume one would wish for, which remains to be produced, will have to be prepared by a single author, rather than by a collection of individuals, each concerned primarily with his or her own specialty.

JOHN C. MARR

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(R23)

The Pathology of Fishes. *William E. Ribelin and George Migaki. [ed.] University of Wisconsin Press, Madison, Wis. 1975. xii + 1004 p. $35.00.*

Prior to the publication of this text, there were no detailed reference texts that specifically dealt with the subject of pathology of fishes. This book includes discussion of gross, microscopic, and comparative study of normal and pathological responses of fish to specific disease agents within a single reference. Most of the standard older texts currently employed are the works of single authors that have been translated and require updating of the material. In sharp contrast, this book represents the combined effort of leading authorities presenting the latest information in their direct presentations.

Each chapter deals with a specific disease consideration, including a chapter on normal comparative structure of fish. Discussions of disease agents include specific infections and parasitic diseases, diseases of specific species of fish and their organ systems, intoxications, and metabolic and nutritional diseases, as well as tumors of fish.

The student who wishes an introduction to the subject of fish pathology and the professional expert who wishes a detailed bibliography of current selected references will find this book invaluable. For those whose interest is in comparative pathology, the text is unique. Each chapter has a "question and answer" section that stimulates the author to respond to additional considerations that give new insights to the reader.

This book is limited to the subject of fish pathology. For those who are primarily interested in the prevention, treatment, or control of fish diseases, this is not the book of choice. While the price of the book is substantial, it is justified in this inflationary period by the fine quality of paper, printing and rich illustrations. This book is a valuable addition to any library related to fish pathology.

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Books Received/Livres reçus

December 1975–January 1976

***Black Bass Biology and Management.** *Edited by Henry Clepper. Sport Fishing Institute, 608 Thirteenth St., N.W., Washington, D.C. 20005, 1975. x + 534 p. $20 paper, $25 cloth. (Available on direct prepaid order, only from above address.)*
(Proceedings of the First National Bass Symposium sponsored by the Sport Fishing Institute at Tulsa, Oklahoma, Feb. 3–6, 1975. Major sections of the book cover life histories and biology of centrarchid basses, community structure, population dynamics, smallmouth bass in Courtois Creek, influence of environmental parameters on basses, culture, management practices, and identification of problems and needs in management and research.

***Control of Fish Quality.** *J. H. Connell. Fishing News (Books) Ltd., West Byfleet, Surrey, England, 1975. xi + 179 p. £6.50.*
(Account of fish and fishery product quality and measures that can be taken to regulate or control adverse changes in it.)

***Lake Kariba. A Man-Made Tropical Ecosystem in Central Africa.** *Edited by E. K. Balon and A. G. Coche. Dr. W. Junk b.v. Publishers, The Hague, 1974. xii + 767 p. $90.*
(Presents data on limnology and fish production in one of the world's largest man-made lakes.)

***Modelling of Marine Systems.** *Edited by Jacques C. J. Nihoul. Elsevier Scientific Publishing Company, Amsterdam, Oxford, New York, 1975. xix + 272 p. $29.25.*
(Surveys state of the art and the concepts and techniques used in marine modelling. Conclusions and recommendations from the NATO Conference on Marine Modelling, Portugal, 1973, are included and provide a guideline for future work.)

Should Trees Have Standing? Toward Legal Rights for Natural Objects. *Christopher D. Stone. Foreword by Garrett Hardin. William Kaufmann, Inc., Los Altos, California, 1974. xvii + 102 p.*
(Essay proposing that natural objects should have legal rights. The U.S. Supreme Court's opinions in the Mineral King–Disney–Sierra Club suit are included in full.)

***The Study of Benthic Communities.** *Robert H. Parker. Elsevier Scientific Publishing Company, Amsterdam, Oxford, New York, 1975. x + 279 p. $32.50.*
(Traces most energy pathways through a small tidal marine ecosystem. Multivariate analyses are used to correlate over 130 variables dealing with the environment and animals, and boundaries of animal communities are then defined.)

***Temperature–Salinity Analysis of World Ocean Waters.** *O. I. Mamayev. Translated from Russian by Robert J. Burton. Elsevier Scientific Publishing Company, Amsterdam, Oxford, New York, 1975. x + 374 p. $43.75.*
(Exhaustive treatment of theoretical and practical aspects of temperature–salinity analysis as used in oceanography. Analytical theory of T–S curves, properties of the diagram as an analytical tool, and techniques of thermohaline analysis are discussed.)

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*To be reviewed.

COMING NEXT MONTH / À PARAÎTRE LE MOIS PROCHAIN

Test of a model for predicting the body burden of trace contaminants in aquatic consumers
J. W. Elwood and L. D. Eyman

Hyponeustonic fish larvae in the Northumberland Strait during summer 1962 **D. J. Faber**

Thermal requirements of the early life history stages of walleye, *Stizostedion vitreum vitreum*, and sauger, *Stizostedion canadense* **W. M. Koenst and L. L. Smith Jr.**

Parasites as potential biological tags of Atlantic salmon (*Salmo salar*) smolts in the Miramichi River system, New Brunswick **G. M. Hare and M. D. B. Burt**

Antimycin: uptake, distribution, and elimination in brown bullheads (*Ictalurus nebulosus*)
D. P. Schultz and P. D. Harman

Effects of environmental disturbance on the distribution and primary production of benthic algae on a British Columbia estuary **W. M. Pomeroy and J. G. Stockner**

Ontogenetic variation in the multiple hemoglobins of coho salmon (*Oncorhynchus kisutch*) and the effect of environmental factors on their expression **M. A. Giles and W. E. Vanstone**

Fecundity of coho salmon (*Oncorhynchus kisutch*) from the Great Lakes and a comparison with ocean salmon **T. M. Stauffer**

Lipid composition of slender tuna (*Allothunnus fallai*) as related to lipid composition of their feed (*Nyctiphanes australis*) **D. G. Bishop, D. G. James, and J. Olley**

Notes

Survival of *Daphnia*, crayfish, and stoneflies in air-supersaturated water **A. V. Nebeker**

Viral hemorrhagic septicemia: comparative susceptibility of rainbow trout (*Salmo gairdneri*) and hybrids (*Salmo gairdneri* × *Oncorhynchus kisutch*) to experimental infection
W. M. Ord, M. Le Berre, and P. de Kinkelin

Application of 24-h postexposure observation to acute toxicity studies with invertebrates
C. R. Hansen Jr. and J. A. Kawatski

Migrating Atlantic salmon (*Salmo salar*) caught by otter trawl on the Newfoundland continental shelf
W. H. Lear

Acute toxicities of spruce and hemlock bark extracts to some estuarine organisms in southeastern Alaska
D. V. Buchanan, P. S. Tate, and J. R. Moring

A further note on sampling trawl catches at sea **A. J. Westrheim**

Survival and growth of rainbow trout (*Salmo gairdneri*) in four prairie lakes, North Dakota
G. L. Myers and J. J. Peterka

Infectious pancreatic necrosis: transmission with iodine-treated and nontreated eggs of brook trout (*Salvelinus fontinalis*) **G. L. Bullock, R. R. Rucker, D. Amend, K. Wolf, and H. M. Stuckey**

Perspectives

Science for the scattered fisheries of the Canadian interior **H. A. Regier**

Smolt transformation: evolution, behavior, and physiology **W. S. Hoar**

Letters/Lettres

Some comments on Balon's terminology of fish development intervals **W. J. Richards**

A note concerning Dr Richards' comments **E. K. Balon**

Book Reviews/Comptes rendus

Handbook of trout and salmon diseases (R. J. Roberts and C. J. Shepherd) **T. P. T. Evelyn**

Journal of the Fisheries Research Board of Canada

Guide for Authors

A more detailed guide on preparation of manuscripts from theses is available from the Office of the Editor.

Content

We invite submission of manuscripts in the broad field of fisheries management, ocean science, and aquatic environments relevant to Canada.

Articles: Reports of research studies of broad scope that are original contributions to science.

Notes: Differ from papers on the basis of scope or completeness, rather than quality of research. They may be on problems with narrow, readily defined limits; on work that is largely confirmatory; on methodology or research techniques; on advances in knowledge arising as by-products of broader studies; on progress in projects that were discontinued; or on preliminary findings that warrant publication before problems are resolved. They may be organized with as much flexibility as articles except that they have no centered headings and should occupy no more than four printed pages.

Reviews: Detailed articles that are critical appraisals of broad areas of investigation, particularly in fields that have shown rapid development in recent years.

Perspectives: Essays of opinion or hypothesis on fisheries, ocean science, aquatic environments, or related topics that are of concern to the professional and layman. References are desirable; informal style is welcomed.

Letters: Opinion on topics recently under discussion in the *Journal*; preferred maximum length, 500 words. The Editor reserves the right to shorten letters and to make other changes to accord with the style of the *Journal*.

Editorial Practices

The *Style Manual for Biological Journals* (3rd ed. American Institute of Biological Sciences, 3900 Wisconsin Ave. NW, Washington, D.C.) is used as the authority in matters of form. The standards for spelling are: *Webster's Third New International Dictionary* and *le Grand Larousse Encyclopédique*. Titles of periodicals are abbreviated as in the *Biological Abstracts* list. References to literature are made by the name-and-year system. The *Journal* follows the names and spelling recommended in *A List of Common and Scientific Names of Fishes from the United States and Canada* (3rd ed. 1970), Special Publication No. 6 of the American Fisheries Society.

Series titles should be avoided. An *abstract* is required for all manuscripts, and should state concisely, in less than 250 words, what was done, the essentials of the new knowledge, and the main conclusions. Abstracts are published in English and French; those submitted in one language only are translated by the *Journal*.

The Editor normally submits each manuscript to at least two *referees*. Contributors are encouraged to suggest at least four names to be added to the list from which referees may be selected.

Illustrations should be submitted as photographic reproductions no larger than a standard page and must accompany the manuscript. Prints should not be mounted, stapled, or clipped. Symbols, letters, and numerals must be neat, and at least 1.5 mm high when the illustration is reduced to the appropriate size. Drawings should, when possible, be grouped to facilitate comparisons.

Proofs are sent to the author, who is responsible for correcting only errors in typesetting. *Alterations to the content must be avoided.*

Fifty *reprints* are supplied free. Additional reprints may be purchased at the time of publication (an order form is sent out with the galley proof). All orders should be sent to the Printing Department, University of Toronto Press, 5201 Dufferin Street, Downsview, Ont. M3H 5T8. Invoices relating to the reprints will be sent out by the Printer. A charge of $40.00 per page is made for articles published. When submitting a manuscript, an author should state that his institution will accept this charge.

Checklist on Manuscripts

Attention to the following questions will expedite appraisal of manuscripts by referees and editors.

Are the findings, interpretations, and conclusions adequately documented and relevant to the purpose of the study?

Are all the tables and figures needed, and organized to facilitate comparisons? Are there inconsistencies between tables or figures and the text, or within the text? Should some of the data be made available separately, in a manuscript report or at a data repository? If you use statistical analysis are the statistics subordinate to the research?

Would any of the text be clearer if condensed? Are summary statements given at the beginning of sections and paragraphs, and are details in sections and paragraphs relevant to their topics? Does the organization of the manuscript follow logically from the statement of purpose in the introduction?

Does the *title* encompass the content of the report?

Does the *abstract* give the essentials of the new knowledge?

Is the *introduction* largely limited to the scope, purpose, and rationale of the study? Is review of the literature limited to defining the problem?

Are details of *materials and methods* limited to what scientists need in understanding the design of the study and in judging adequacy of the data?

Are generalizations on the *results* adequate? Are findings distinguished from inferences from them?

Is *discussion* limited to interpretation and significance of the findings?

Journal de l'office des recherches sur les pêcheries du Canada

Guide des Auteurs

On peut se procurer au bureau du Directeur de la publication un guide plus détaillé pour la préparation de manuscrits à partir de thèses.

Sujets

Nous désirons reçevoir des manuscrits s'insérant dans le vaste domaine de la gestion des pêches, des sciences de la mer et des milieux aquatiques au Canada.

Articles: Comptes rendus d'études d'une portée générale, constituant des contributions inédites à la science.

Notes: Se distinguent des articles par la portée et le degré d'achèvement plutôt que par la qualité des recherches. Elles peuvent traiter de problèmes particuliers et bien définis; de travaux qui sont en grande partie confirmatoires; de méthodologie ou de techniques de recherche; de progrès de la connaissance découlant d'études plus vastes; de développements dans des projets abandonnés; ou de découvertes préliminaires qui méritent d'être publiées avant la solution de problèmes à l'étude. Les notes peuvent être présentées d'une façon aussi souple que les articles, mais elles ne doivent pas porter d'en-têtes centrés ni occuper plus de quatre pages.

Critiques: Articles détaillés constituant une appréciation critique de vastes champs de recherches, particulièrement dans les domaines ayant connu de rapides progrès au cours des dernières années.

Perspectives: Essais de l'opinion ou de l'hypothèse sur les pêches, les sciences de la mer, les milieux aquatiques, ou sur des sujets connexes qui intéressent professionnels et profanes. Il est préférable de donner des références et d'écrire, si possible, dans un style simple.

Lettres: Opinions sur des sujets exposés dans le *Journal*. Longueur maximale préférable: 500 mots. Le directeur se réserve le droit de raccourcir les lettres et de faire d'autres modifications dans le style du *Journal*.

Dispositions générales

Le *Style Manual for Biological Journals* (3e éd., publié par l'American Institute of Biological Sciences, 3900 avenue Wisconsin N.O., Washington, D.C.) sert de guide quant à la forme de présentation. Le *Webster's Third New International Dictionary* et le *Grand Larousse Encyclopédique* font autorité pour l'orthographe des mots. Les titres de périodiques sont abrégés suivant les *Biological Abstracts*. Les références bibliographiques dans le texte doivent suivre le système nom de l'auteur-année. Pour les noms de poissons et leur orthographe, le *Journal* suit les recommandations contenues dans *A List of Common and Scientific Names of Fishes from the United States and Canada* (Troisième édition, 1970), Publication spéciale No. 6 de l'American Fisheries Society.

Il faut éviter les *articles en série*. Un *résumé* doit accompagner chaque texte et doit exposer brièvement (moins de 250 mots) le travail accompli, les points essentiels des connaissances acquises et les principales conclusions. Les résumés paraissent en anglais et en français, le *Journal* veillant à la traduction dans le cas des présentations unilingues.

Le directeur soumet d'ordinaire chaque texte à l'approbation de deux *arbitres* ou plus. Nous encourageons les collaborateurs à suggérer au moins quatre personnes dont les noms pourraient figurer sur la liste des arbitres.

Les *illustrations* doivent être sous forme de reproductions photographiques ne pas dépasser les dimensions d'une page normale et accompagner le texte. Elles ne doivent pas être montées, coupées ni brochées. Les symboles, lettres ou chiffres doivent se détacher avec précision et avoir au moins 1,5 mm de hauteur lorsque l'illustration est réduite à la grandeur voulue. Les dessins doivent, autant que possible, être groupés pour faciliter l'étude comparative.

L'auteur doit revoir les *épreuves* qu'on lui remet et en corriger seulement les erreurs typographiques. *Il doit éviter de changer le contenu du texte.*

Cinquante *tirés à part* sont fournis gratuitement. Les tirés à part peuvent être achetés au moment de la publication. Un bon de commande à cet effet accompagne les épreuves; il doit être retourné en même temps que celles-ci, non pas au bureau de la rédaction, mais au Printing Department, University of Toronto Press, 5201 Dufferin Street, Downsview, Ont. M3H 5T8, qui se chargera de la facturer. Les frais sont de $40.00 par page pour la publication des articles. Quand un auteur soumet un manuscrit, il doit spécifier que son institution accepte les frais.

Listes de contrôles pour manuscrits

Une attention particulière aux questions suivantes facilitera l'évaluation des manuscrits par les arbitres et les éditeurs.

Les découvertes, interprétations et conclusions sont-elles bien documentées et pertinentes au but de l'étude?

Les tableaux et figures sont-ils tous nécessaires et agencés pour faciliter les comparaisons? Y a-t-il contradiction entre les tableaux ou les figures et le texte, ou dans le texte même? Est-ce que certaines données devraient être présentées séparément, dans un rapport manuscrit ou dans un dépôt de données? Si vous faites une analyse statistique, la statistique cède-t-elle le pas à la biologie?

Le texte y gagnerait-il en clarté s'il était plus condensé, en tout ou en partie? Contient-il des exposés sommaires au début de chaque section ou paragraphe, et les détails de ces sections et paragraphs son-ils pertinents au sujet traité? La marche du manuscrit est-elle conforme à la logique énoncée dans l'introduction?

Le *titre* est-il concis, tout en étant représentatif du contenu du rapport?

Le *résumé* donne-t-il l'essentiel des connaissances nouvelles?

L'*introduction* se limite-t-elle à une esquisse de la portée, du but et des raisons de l'étude? La revue de la littérature se limite-t-elle à la définition du problème?

La section *matériel et méthode* contient-elle le minimum de détails qui permettront aux autres scientifiques de saisir les grandes lignes de l'étude et de juger de la valeur des données?

Les généralisations sur les *résultats* sont-elles adéquates? Permettent-elles de distinguer entre constatations et déductions?

La *discussion* est-elle limitée à l'interprétation et à l'évaluation des découvertes?

Journal of the Fisheries Research Board of Canada

Guide for Typists Preparing Copy

Read "Guide for Authors" in the current volume of the *Journal*. When in doubt about how to type or arrange details, follow *Journal* practice.

Type the manuscript on white bond paper, not flimsy, preferably size 21.5 × 28 cm (8.5 × 11 inches). If possible, use paper with the lines numbered in the margin. Leave all margins at least 2.5 cm wide.

Double-space all parts of the manuscript, including tables, captions for illustrations, footnotes, and the reference list. Underline only material that is to be printed in italics. Indent the first line of each paragraph in the text and of each footnote.

In titles and headings, capitalize the first letter of each word. For small capitals, use small letters.

Indicate all text footnotes by superscript arabic numerals. Number them consecutively throughout the manuscript, starting with those on the title page. Place each footnote immediately below the reference to it, separating it from the text by horizontal lines.

On the first page type only the title, the authors' names and affiliations, related footnotes, and the running head (see Appendix A). Type the abstract on a separate page (see Appendix B). Number all pages, beginning with the title page and including those for tables and captions for illustrations.

Have the author check the finished manuscript, and send the ribbon copy and two other clear copies to the Office of the Editor.

Tables: Examine the current volume of the *Journal* to see how tables are set up (see Appendix C).
Note especially the following concerning *numbered tables:*

Type each on a separate page, using paper without the lines numbered.

Number them with arabic numerals.

Use horizontal lines above and below the headings and below the columns, and seldom elsewhere. Never use vertical lines; leave extra spaces instead.

Indicate table footnotes by superscript small letters and type them below the table.

Place the tables after the list of references.

Where each table is first referred to, type in the margin "Table — near here."

Note that *text tables* are not numbered, are typed with the text, and seldom need horizontal lines (see Appendix C).

Illustrations: Send a photographic reproduction (no larger than 21.5 × 28 cm) of each illustration with the manuscript. Type the author's name and figure number on a strip of paper and fasten it to the back of the print. Do not write or type on the print itself. Also, type the captions in succession on a separate page, headed "Captions for illustrations." Put this page at the end of the manuscript.

Where each figure is first referred to, type in the margin "Fig. — near here."

Mathematical Expressions, Equations, and Formulas: Leave three spaces above and below lines that have much material in superscripts, and above and below equations and formulas. In the various expressions, place each component in its correct position (see Appendix D).

Show all symbols clearly. In the margin, spell out Greek letters the first time they occur, and distinguish between the small letter *l* and the numeral *one*, and between the capital letter *O* and the numeral *zero*. Make other explanatory notes similarly.

References: Check the text to see that all references in the list are referred to. Type the reference list, double-spaced, in the style used in the *Journal* (see Appendix E). Names of journals are abbreviated as in the *Biological Abstracts* list; if an abbreviation is in doubt, type the name in full.

Spellings: About 100 words common in fisheries journals are listed in Appendix F.

Appendices: Appendices A to F are available on request from the Office of the Editor.

Journal de l'office des recherches sur les pêcheries du Canada

Guide pour les secrétaires d'auteurs

Lisez le "Guide des Auteurs" reproduit dans l'édition courante du *Journal*. Si vous avez des doutes sur la façon de dactylographier le texte ou d'en agencer les détails, suivez la pratique du *Journal*.

Dactylographiez le manuscrit sur papier blanc de bonne qualité, et non sur papier pelure, mesurant de préférence 21,5 × 28 cm (8,5 × 11 pouces), laissant toujours une marge d'au moins 2,5 cm. Si possible, employez un papier avec lignes numérotées en marge.

Tout le manuscrit, y compris tableaux, légendes de figures, renvois et références bibliographiques, devra être dactylographié à double interligne. Ne soulignez que les mots qui devront être imprimés en italique. Mettez en retrait la première ligne de chaque paragraphe du texte et de chaque renvoi.

Dans les titres et les en-têtes, n'employez la majuscule que pour le premier mot et les noms propres. Pour les petites majuscules, employez les miniscules du dactylographe.

Indiquez tous les renvois du texte par des chiffres arabes suscrits. Numérotez les renvois consécutivement par tout le texte, à commencer par ceux de la page titre. Placez chaque renvoi directement sous sa référence, le séparant du texte par des lignes horizontales.

La première page ne doit contenir que le titre de la publication, le nom et l'affiliation des auteurs, les renvois appropriés et le titre courant (voir Appendice A). Dactylographiez le résumé sur une page séparée (voir Appendice B). Numérotez chaque page, à commencer par la page titre, et y compris celles qui contiennent les tableaux et la légende des figures.

Voyez à ce que l'auteur vérifie le manuscrit final, et soumettez la première copie ainsi que deux copies lisibles au bureau du Directeur de la publication.

Tableaux. Consultez une copie du *Journal* pour la composition des tableaux (voir Appendice C).

Portez une attention particulière à ce qui suit au sujet des *tableaux numérotés*.

Dactylographiez chaque tableau sur feuille séparée, vous servant de papier sans lignes numérotées.

Numérotez les tableaux en chiffres arabes.

Tracez des lignes horizontales au-dessus et au-dessous des en-têtes et au-dessous des colonnes, rarement ailleurs; ne tracez jamais de lignes verticales; laissez plutôt des espaces supplémentaires.

Indiquez les renvois dans les tableaux par les minuscules suscrites et placez les renvois au bas du tableau.

Placez les tableaux à la suite des références bibliographiques.

A l'endroit où le tableau est mentionné pour la première fois, indiquez dans la marge "Tableau — près d'ici."

Remarquez que les *tableaux dans le texte* ne sont pas numérotés, sont dactylographiés avec le texte et ne requièrent que rarement des lignes horizontales (voir Appendice C).

Figures: Envoyez avec le manuscrit un reproduction photographique (21,5 × 28 cm ou moins) pour chaque illustration. Dactylographiez le nom de l'auteur et le numéro de la figure sur une lisière de papier que vous collerez au dos. N'écrivez pas à la main ou à la machine sur la photographie même. En plus, dactylographiez les légendes, dans l'ordre, sur feuille séparée, intitulée "Légendes des figures." Intercalez cette feuille à la suite du texte.

A l'endroit où chaque figure est mentionnée pour la première fois, inscrivez en marge "Fig. — près d'ici."

Equations, formules et expressions mathématiques: Laissez trois espaces au-dessus et au-dessous des lignes renfermant des chiffres ou des lettres suscrits et souscrits, ou encore au-dessus et au-dessous d'équations et de formules. Placez chaque composante des différentes expressions dans la position qui lui est propre (voir Appendice D).

Indiquez clairement tous les symboles. Epelez en marge les lettres grecques, la première fois qu'elles apparaissent. Faites une distinction entre la lettre *l* et le chiffre *un*, et entre la majuscule *O* et le chiffre *zéro*. Insérez de la même façon toute autre note explicative.

Références: Vérifiez le texte afin de vous assurer que toutes les références apparaissant dans la liste bibliographique sont bien mentionnées dans le texte. Dactylographiez à double interligne dans le style en usage dans le *Journal* (voir Appendice E). Les noms de revues sont abrégés suivants les *Biological Abstracts*; en cas de doute, écrivez le nom au long.

Orthographe: Une liste d'environ 100 mots communément employés dans les périodiques sur les pêches est donnée en Appendice F.

Appendices: On peut se procurer une copie des Appendices A à F sur demande au bureau du Directeur de la publication.

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